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EUROPEAN CHEMICALS AGENCY

Guidance on the Biocidal Products Regulation

Volume II: Efficacy
Parts B+C: Assessment and Evaluation
Version 6.0, August 2023

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Guidance on the BPR: Volume II Efficacy - Assessment and Evaluation (Parts B+C)

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DOCUMENT HISTORY

Version	Comment	Date
Version 1.0	First edition	February 2017
Version 2.0	Update to PT8 The text has been revised as follows: • To add a new Appendix 12; • To revise section 5.5.8.3 to remove temporary footnote re. the new Appendix 12; • To add a new footnote to section 5.5.8.2.2.3, Use Class 2, Test Species; • To re-number Appendices after the new Appendix 12 and revise all cross references to these Appendices.	December 2017
Version 3.0	Update to PT5 Drinking Water Disinfectants: • Section 5.4.5 has been revised and updated; • Appendix 3 has been revised for PT5; • Appendix 4 has been revised for PT5. In addition corrections and editorial revisions have been made as follows: • Section, 5.5.8.2.2.3: the footnote added in version 2.0, (footnote 28) has been moved to the beginning of the section because it applies to Use Class 1 as well as Use Class 2. It is now footnote 24; • Appendix 3: corrections are made to: ○ *Escherichia coli* ATCC 10536: amend O to (X) for PT2 and PT3; ○ *Escherichia coli K12 NCTC 10538*: amend X to (X) for PT2; ○ Addition of *Proteus vulgaris* ATCC 13315 (not for teat disinfection) for PT3. • Appendix 4: the tables that were on the ECHA Biocides webpages, have been moved into the Guidance document because the revised information in the tables is directly linked and reflected in the guidance text and the "Notes" are giving guidance not simply test methodology details; • Appendix 4: corrections are made to: ○ Corrections to Guidance section cross references for PT4 - surfaces in drinking water systems. ○ Correction of loq reduction in EN 13623 to 4 • Appendix 16: a note has been added to inform readers that this appendix contain information that	April 2018

	refers to the BPD and which is now obsolete and should also refer to the text in sections 2 and 5; this will be revised at the next update.	
Version 4.0	Update to: List of Abbreviations. PT18 - Section 5.6.4 - Insecticide, Acaricides & other Biocidal Products against Arthropods+ PT 19 Repellents & Attractants (arthropods): • The title has been amended to *Insecticides, acaricides and products to control other arthropods*; • All information related to attractants and repellents has been removed from section 5.6.4; PT19 - Section 5.6.5 PT19 Repellents and attractants has been added; Appendix 17: Table 40: PT 19 – Repellents & Attractants has been removed. Appendix 18: has been updated with relation to PT19.	December 2021
Version 4.1	Corrigendum to remove the remaining text concerning repellents and attractants (except information in section 5.6.4.5 Termites) from section 5.6.4 PT18 Insecticides, acaricides and products to control other arthropods.	February 2022
Version 5.0	Update to • Section 5.4.0.4.5 'Co-formulant(s) being a potential active substance' has been added. • Section 5.4.1.2.2, Vaccinia virus as test organism has been added; PT2 • Section 5.4.2.2.4, information about differentiation of virucidal claims has been added; • Section 5.4.2.3.2, information about: ○ Phase 2, step 2 test used for the respective application methods has been added; • Section 5.4.2.5 Room disinfection/automated airborne disinfection of surfaces has been updated: ○ requirement for a quantitative suspension test (phase 2, step 1) has been removed; ○ parameters used in efficacy tests and the parameters to be taken into account in the SPC have been revised; ○ requirements for biological and chemical validation in room disinfection have been added; • Section 5.4.2.8 Air-conditioning systems – the requirement for a quantitative suspension test (phase 2, step 1) has been removed; • Section 5.4.2.10 Textile/laundry process disinfection	November 2022

	has been updated: ○ information about products used as disinfectants in combination with detergents has been added; ○ the recommended test organisms have been added; PT3 • Section 5.4.3.2 and 5.4.3.7, recommendation for DVG guidelines has been added; • Section 5.4.3.8 on Room disinfection/automated airborne disinfection of surfaces has been added. PT4 • Section 5.4.4.1 has been amended in order to give a possibility to differentiate the contact time and dose for bacteria and yeasts for professional users; • Section 5.4.4.3. on room disinfection/automated airborne disinfection of surfaces has been added; • Section 5.4.4.7 on disinfection of inner surfaces in human drinking water systems, the requirements for phase 2, step 2 tests have been added; • Section 5.4.4.9 on other uses in PT4 has been updated about requirements concerning disinfection of packaging before aseptic filling. PT5 • Section 5.4.5.2 on disinfection at the drinking water suppliers and their water distribution systems has been updated about information of test requirements for chlorine-based disinfectants. Appendix 1 (Table 5) is updated to reflect the changes made in Appendix 4. Appendices 2, 3 and 4: the test requirements and test organisms are updated based on EN standards that have been published after Vol II Parts B+C Ver 3.0 was published (EN 1276:2019. EN 1650:2019, EN 1656:2019, EN 14476:2013+A2:2019, EN 17126:2018, EN 16777:2018, EN 17111:2018, EN 17122:2019, EN 17272:2020, EN 17387:2021). The main guidance text has been updated accordingly, where relevant. In addition some corrections and editorial revisions have been made to both appendices. In addition corrections revisions have been made as follows: • Harmonising the abbreviation of litre into l (as in the BPR) • Harmonising the abbreviation of colony forming units into cfu • Harmonising the abbreviation of logarithmic into lg	

	(instead of log) In addition, some smaller editorial corrections for aligning the spelling have been made.	
Version 6.0	Update to: PT11 - Section 5.5.11 PT11 Preservatives for liquid-cooling and processing systems has been added PT12 - Section 5.5.12 PT12 Slimicides has been added Appendix 25 has been added. Appendix 26 has been added. Appendix 8 has been updated by adding the commonly used methods for PT11 and 12 Corrigendum to: • Appendix 3 (the Key for Table 42 section has been corrected) • Appendix 4: PT 3 Teat disinfection (yeasts pre/post-milking - phase 2, step 2 test requirement has been changed from "If claimed" to "Optional") In addition, some smaller editorial corrections for aligning the spelling have been made.	August 2023

PREFACE

The Guidance on the Biocidal Products Regulation (BPR) is to be applied to applications for active substance approval and product authorisation as submitted from 1 September 2013, the date of application (DoA) of the Biocidal Product Regulation (the BPR).

This document describes the BPR obligations and how to fulfil them.

The scientific guidance provides technical scientific advice on how to fulfil the information requirements set by the BPR (Part A) and how to assess and evaluate the efficacy to establish the benefit arising from the use of biocidal products and to prove that it is sufficiently effective (Parts B+C).

In addition to the BPR guidance, the Biocidal Products Directive (BPD) guidance and other related documents are still considered applicable for new submissions under the BPR in the areas where there are no BPR guidance or it is under preparation. Furthermore these documents are still valid in relation to the applications for active substance approval submitted under the BPD that may still be under evaluation. Also the Commission has addressed some of the obligations in further detail in the Biocides Competent Authorities meetings documents which applicants are advised to consult. Please see ECHA Biocides Guidance website for links to these documents: [https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation].

Applicability of Guidance

Guidance on applicability of new guidance or guidance related documents for active substance approval is given in the published document "*Applicability time of new guidance and guidance-related documents in active substance approval*" available on the BPC Webpage[1] and for applicability of guidance for product authorisation, please see the CA-document CA-july2012-doc6.2d (final), available on the ECHA Guidance page [CA-July12-Doc.6.2.d - Relevance of new guidance].

[1] Link available under Working Procedures (right column) [https://echa.europa.eu/about-us/who-we-are/biocidal-products-committee]

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NOTES to the reader:

In this document text cited from the Biocidal Products Regulation (EU) No 528/2012 is indicated in green boxes.

- This symbol highlights text to be noted.

Section 5.6 and sub-sections for PT10, PT15, PT16, PT17 and PT20: please refer to the General sections 1-3 of this guidance and the TNsG.

List of Abbreviations

Abbreviation	Explanation
AACC International	American Association of Cereal Chemists New name: Cereals & Grains Association https://www.cerealsgrains.org
AATCC	American Association of Textile Chemists and Colorists https://www.aatcc.org
AFNOR	French Association for Standardisation (Association Française de Normalisation) http://www.afnor.org
AFPP	Association Française de Protection des Plantes New name: Vegephyl - Association for plant health https://www.vegephyl.fr
AOAC	Association of Official Analytical Collaboration (AOAC) INTERNATIONAL http://www.aoac.org
AS	Active substance
ASTM	American Society for Testing Materials (ASTM) INTERNATIONAL http://www.astm.org
ATCC	American Type Culture Collection http://www.lgcstandards-atcc.org
BBA	Federal Research Centre for Cultivated Plants (Julius Kühn-Institut - Bundesforschungsinstitut für Kulturpflanzen) https://www.julius-kuehn.de
BP	Biocidal product
BPD	Biocidal Products Directive 98/8/EC
BPR	Biocidal Products Regulation (EU) No 528/2012
BSI	British Standard Institution (BS standards) https://www.bsigroup.com
CA	Competent Authority ○ Evaluating CA (eCA) is the Competent Authority that evaluates the application for an active substance approval or an application for a Union authorisation. ○ Receiving CA is the Competent Authority that receives an application for a National Authorisation.
CAR	Competent Authority Report
Cefic	European Chemical Industry Council https://cefic.org/
CEN	European Committee for Standardisation http://www.cen.eu

Abbreviation	Explanation
CEPE	European Council of the Paint, Printing Ink, and Artist's Colours Industry https://www.cepe.org/
cfu	Colony forming unit
CIP	Cleaning-in-Place
CSMA	Chemical Specialties Manufacturers Association
CT	Contact time
CTBA	Technical Center for Wood and Furniture New name: Technological Institute (L'institut technologique FCBA) https://www.fcba.fr
Ctgb	Board for the Authorisation of plant protection products and biocides (Netherlands) https://www.ctgb.nl
CV	Critical value
DG SANCO	Directorate-General Health and Consumer Protection New name: Directorate-General for Health and Food Safety (SANTE)
DIN	German Institute for Standardization (Deutsches Institut für Normung) http://www.din.de/
DVG	German Veterinary Medical Society (Deutsche Veterinärmedizinischen Gesellschaft) http://www.dvg.net
ECDC	European Centre for Disease Prevention and Control
EMA	European Medicines Agency https://www.ema.europa.eu/
EN	European Standard
EPA	United States Environmental Protection Agency https://www.epa.gov/
EPPO	European and Mediterranean Plant Protection Organization https://www.eppo.int
ESL	Estimated service life
EU	European Union + Norway, Iceland and Lichtenstein Please note the BPR applies to the European Economic Area (EEA) and thus all references to the EU in the text should be understood as EEA (EU + Norway, Iceland and Lichtenstein)
FCBA	Technological Institute (L'institut technologique FCBA) https://www.fcba.fr/
GLP	Good laboratory practice

Abbreviation	Explanation
ISO	International Organization for Standardisation http://www.iso.org
KD	Knock down
KD_{50}	Knock down for 50% of the group of tested animals
KT_{50}	Knock down time for 50% of the group of tested animals
LD_{50}	Lethal dose for 50% of the group of tested animals
lg	Log reduction (the relative number of living microbes that are eliminated by disinfection)
l	Litre
MS	Malaysian Standard https://ikm.org.my/sda/
OCSPP	Office of Chemical Safety and Pollution Prevention (EPA) https://www.epa.gov/aboutepa/about-office-chemical-safety-and-pollution-prevention-ocspp
OECD	Organisation for Economic Co-operation and Development http://www.oecd.org
OPPTS	Office of Prevention, Pesticides and Toxic Substances New name: Office of Chemical Safety and Pollution Prevention (OCSPP, please see above)
prEN	Draft European Standard
PAR	Product Assessment Report
PT	Product type
SABS	South African Bureau of Standards https://www.sabs.co.za/
SANTE	Directorate-General for Health and Food Safety
RIVM	National Institute for Public Health and the Environment (Netherlands) https://www.rivm.nl/
SPC	Summary of Product Characteristics
TNsG	Technical Notes for Guidance
TVC	Total viable count
UC	Use Class
US EPA	United States Environmental Protection Agency http://www.epa.gov/
VAH	Association for Applied Hygiene (Verbund für Angewandte Hygiene) http://www.vah-online.de/
VOC	Volatile organic compound
DWD	Drinking Water Directive

Glossary of Terms

Standard term	Explanation
Virucidal activity against enveloped viruses (see also Virucidal activity and Limited spectrum virucidal activity)	A claim for hygienic hand and skin disinfectants, and hard surface disinfectants in PT 2, with activity against enveloped viruses only.
Algaecide	A product or active substance used to control (inhibit the growth) or kill algae.
Algaecidal activity	The capability of a product or active substance to produce a reduction in the number of viable algae cells under defined conditions.
Antimicrobial product	A product which prevents the growth of/reduces the number of/mitigates the growth of micro-organisms
Antiseptic	Product – excluding antibiotics – that is used to bring about antisepsis by destroying or inhibiting the growth of microorganisms
Antisepsis	Application of an antiseptic on living tissues causing an action on the structure or metabolism of microorganisms to a level judged to be appropriate to prevent and/or limit and/or treat an infection of those tissues
Bactericide	A product or active substance which irreversibly inactivates vegetative bacteria under defined conditions
Bactericidal activity	The capability of a product or active substance to produce a reduction in the number of viable bacterial cells of relevant test-organisms under defined conditions
Bacteriostatic activity	Capability of a product or active substance to inhibit the growth of bacteria under defined conditions
Biocidal product/ Biocide	BPR Article 3(1)(a): *— any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action,* *— any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action.* *A treated article that has a primary biocidal function shall be considered a biocidal product.*

Standard term	Explanation
Biofilm	An accumulation of microbial cells immobilised on a substratum and embedded in an organic polymer matrix of microbial origin
Biostatic product	A product which inhibits the growth of micro-organisms under defined conditions
Curative effect on biofilm	The biocide is added after the biofilm is formed and acts on biofilm stability, facilitating the biocide interaction with cells – it may or may not act as detergent and detach the biofilm from the surface
Disinfectant within PT 2, 3, 4 and 5	A disinfectant is a product that reduces the number of micro-organisms in or on an inanimate matrix- achieved by the irreversible action of a product, to a level judged to be appropriate for a defined purpose
Disinfection within PT 2, 3, 4 and 5	Disinfection is the reduction of the number of micro-organisms in or on an inanimate matrix- achieved by the irreversible action of a product, to a level judged to be appropriate for a defined purpose
Skin disinfection within PT1	Skin disinfection is the reduction of the number of micro-organisms on skin, achieved by the irreversible action of a product, to a level judged to be appropriate for a defined purpose
Efficacy	The ability of a product or active substance to produce an effect as described in the label claims made for it, when used under actual use conditions.
Flow condition (for biofilm)	Biofilm is formed on supports of different nature placed along a tube or a chamber where the medium (inoculated and/or fresh) is circulated in a closed (reservoir-pump-tubing) or open (reservoir-pump-tubing-outlet) system
Fungicide	A product or active substance which irreversibly inactivates fungi (vegetative mycelia, budding yeasts and/or their spores) under defined conditions
Fungicidal Activity	The capability of a product or active substance to produce a reduction in the number of viable vegetative yeasts cells and mould spores of relevant test organisms under defined conditions
Fungistatic activity	The capability of a product or active substance to inhibit the growth of fungi under defined conditions
Hygienic hand disinfectants	A hygienic hand disinfectant is a hygienic handrub disinfectant or a hygienic hand wash disinfectant
Hygienic handrub disinfectant	product used for post-contamination treatment that involves rubbing hands, without the addition of water, which is directed against transiently contaminating micro-organisms to prevent their transmission regardless of the resident skin flora
Hygienic handwash disinfectant	product used for post-contamination treatment that involves washing hands with water, which is directed against transiently contaminating micro-organisms to prevent their transmission regardless of the resident skin flora

Standard term	Explanation
Limited spectrum virucidal activity (see also Virucidal activity and Virucidal activity against enveloped viruses)	Limited spectrum virucidal activity is a claim for hygienic hand and skin disinfectants and hard surface disinfectants using Adenovirus and Murine Norovirus as test organisms, thus including activity against the test viruses and all enveloped viruses (see Appendix 5).
Log reduction / log_{10} reduction / lg reduction	Reduction presented in a logarithmic scale. Example 1: when a disinfection reduces 10^8 bacteria to 10^2 bacteria, this is a lg reduction of 6. Example 2: when a disinfection reduces 5.10^7 fungal spores to 8.10^3 fungal spores this is a lg reduction of 3.79.
Microbes/micro-organisms	bacteria (including vegetative cells bacterial spores and mycobacteria) fungi (including yeasts, moulds and fungal spores) algae, viruses (including bacteriophages), protozoa (including cysts and other permanent states), etc.
Mycobactericide	A product or active substance which irreversibly inactivates mycobacteria under defined conditions
Mycobactericidal activity	The capability of a product or active substance to produce a reduction in the number of viable mycobacterial cells of relevant test organisms under defined conditions
Neutraliser	A chemical agent or formulation which suppresses the residual activity of an disinfectant within a test but does not inhibit or inactivate micro-organisms
Performance standard	Regulatory or scientific standard for biocides that is either quantitative or qualitative (that may also be specified in the test method) by which a decision is taken on the acceptability of a claim.
Preventive effect on biofilm	The biocide is present before the biofilm is formed and may act both on cell viability and/or on cell adhesion/biofilm maturation
Product type (PT)	Product types (PT) are defined in BPR annex V
Sporicide	A product or active substance which inactivates dormant bacterial spores under defined conditions
Sporicidal activity	The capability of a product or active substance to produce a reduction in the number of viable bacterial spores of relevant test organisms under defined conditions
Sporistatic activity	The capability of a product to inhibit the germination of dormant bacterial spores under defined conditions
Static condition (for biofilm)	Biofilm is formed on supports such as microplates without agitation after an incubation time that depends on the micro-organism considered

Standard term	Explanation
Surgical hand disinfectants	A surgical hand disinfectant is a surgical handrub disinfectant or a surgical hand wash disinfectant
Surgical handrub disinfectant	Product used for preoperative treatment that involves rubbing hands, without the addition of water, which is directed against the flora of micro-organisms on hands to prevent the transmission of micro-organisms into the surgical wound
Surgical handwash disinfectant	Product used for preoperative treatment that involves washing hands with water, which is directed against the flora of micro-organisms on hands to prevent the transmission of micro-organisms into the surgical wound
Treated article	A treated article is any substance, mixture or article which has been treated with, or intentionally incorporates, one or more biocidal products
Tuberculocide	A product or active substance which irreversibly inactivates *Mycobacterium tuberculosis* under defined conditions
Tuberculocidal activity	The capability of a product or active substance to irreversibly inactivate Mycobacterium tuberculosis, demonstrated by the capability to produce a reduction in the number of viable cells of the test organism *Mycobacterium terrae* under defined conditions
Virucide	A product or active substance which irreversibly inactivates viruses under defined conditions
Virucidal activity (see also Limited spectrum virucidal activity + Virucidal activity against enveloped viruses)	The capability of a product or active substance to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions Virucidal activity is a claim for biocidal products using relevant test organisms and thus showing activity against the enveloped and non-enveloped viruses.
Yeasticide	A product or active substance which irreversibly inactivates yeasts under defined conditions
Yeasticidal activity	The capability of a product or active substance to produce a reduction in the number of viable vegetative yeasts cells of relevant test organisms under defined conditions

1. General Introduction

Evaluation and Assessment

The process of evaluation of active substance applications is given in Article 8 (BPR) and the common principles for the evaluation of dossiers for biocidal products (including the representative biocidal product in the context of the active substance approval) is given in Annex VI (BPR).

The evaluating or receiving CA uses the data submitted in support of an application for active substance approval or authorisation of a biocidal product to make a risk assessment based on the proposed use of the (representative) biocidal product. The general principles of assessment are given in Annex VI (BPR) and the evaluation is carried out according to these general principles. The evaluating body will base its conclusions on the outcome of the evaluation and decide whether or not the biocidal (representative) product complies with the criteria for authorisation set down in Article 19(1)(b) and/or whether the active substance may be approved.

Efficacy data are a fundamental component in the regulatory management and decision making process for biocidal products. Efficacy data are required to establish the benefit arising from the use of biocidal products and must be balanced against the risks their use poses to man and the environment.

Authorisation of a biocidal product will only be granted according to Art. 19 (1) b of the BPR if that product is shown to be sufficiently effective.

Even for the requirement to limit the use to the minimum necessary and the general requirement of sustainable use of biocidal products (Art. 17 and 18 BPR), it is crucial that the biocide in questions delivers the expected effect.

The information and data required relevant to the effectiveness of the active substance(s) to be employed in biocidal products are outlined in Annex II, BPR, title 1 No. 6 and 7 and title 2 No 5 and 6. For biocidal products the data required are set out in Annex III, Title 1 No 6 and 7, and title 2, No 6 and 7.

These general sections at the beginning of this guidance, (namely sections 1, 2 and 3), provide a general overview for the efficacy evaluation; the more specific requirements for each Product Type (PT), which must be met and should be followed in the first instance, are described in the later sections.

2. Claims

2.1 Introduction

The evaluation of the efficacy of a biocidal product is carried out in order to determine whether the claims made for the activity of the active substance (within the product) or the product itself, are supported by suitable efficacy data. A claim is the precondition and base for efficacy testing.

Claims should be comprised of the description of the problem and the way it is suggested to be solved by the biocidal treatment. Claims include written information given in for instance an active substance dossier, in the summary of biocidal product characteristics (SPC), on the label of a product or in product-associated leaflets as well as information provided on a formulator’s/distributor’s website. All claims should be consistent.

Claims can range from simple to complex, depending on the activity and benefits the applicant wishes to claim as resulting from the use of the active substance/biocidal product. This should include as a minimum the following information:

- The purpose of the claim (e.g. prevent the destruction of material by insect infestations, disinfect surface);
- The function of the product (e.g. insecticide, wood preservative, disinfectant, etc.);
- The (group of) target organisms which will be controlled;
- The in-use concentration;
- The use conditions (e.g. contact time, temperature, etc., and area of use);
- The effect which will result from using the product on the target organisms (e.g. kill, control, repel, prevent, etc.);
- Any products, organisms or objects to be protected.

Some examples are available in the different claim matrices and PT-specific guidance sections.

However this basic information can be supplemented by additional claims which further describe the effects of the active substance/product where appropriate, such as:

- How quickly the effect takes place;
- The duration of the effect (residuality) or life-span;
- The types of surface on which the product can be used (e.g. hard porous and non-porous surfaces, softwood).

For products used to treat articles, additional information should be provided:

- Durability of the effect in relation to the expected life-span of the treated article;
- Resilience towards ageing, weathering or other use conditions as for instance washing;
- Where relevant, leaching/migration data for different materials or different use conditions.

All claims made should be supported by data or a suitably robust scientifically based reasoned case.

2.2 Label claims and directions for use

The directions for use and the claims made for the biocidal product are included in a summary of biocidal product characteristics (SPC) in accordance with Article 22(2) (BPR).

A label claim is information which is provided to the user which describes the biocidal effects that will result from using a biocidal product under its normal conditions of use (e.g. when it is used at the recommended dose/application rate, by the recommended application method(s) and in the appropriate areas, etc.). The product label can only include claims that are in line with the authorised uses, as given in the SPC.

Label claims should be as specific as possible, or if more general claims (such as "fast acting") are made, then they should be further clarified on the label where possible (e.g. "fast acting – acts within 5 minutes"). If no clarification is provided, the evaluating Competent Authority should ask the applicant to specify the claim. A judgement as to what a normal user would reasonably expect from the claim should be made. Evaluation should be made according to this claim and the directions for use should be taken into account.

An application for a product authorisation must include a draft SPC.

Applications for product families should include the entire range of the claims proposed for the products within the family.

3. General considerations for the development and reporting of efficacy data

3.1 Efficacy

Efficacy is defined as the ability of a product to fulfil the claims made for it when used according to the directions for use on the proposed product label (as given in the SPC): Is the product actually sufficiently effective against the claimed organisms under the conditions specified? The applicant must provide sufficient information to clearly specify the field of use of the product. In addition, studies must be provided to demonstrate that the product, when used in accordance with the use instructions (concentration, application method, etc.), is sufficiently effective.

3.1.1 Efficacy tests

The applicant must submit studies which clearly demonstrate the efficacy of the active substance/product.

We distinguish various types of studies:

- Screening tests
- Laboratory studies
- Simulated-use tests
- Field trials

Screening tests are usually not related to practical/field conditions and are often not implemented with the complete product but only with the active substance. Such tests are therefore primarily useful for providing supplementary information, for example to demonstrate that the concentration used is optimal.

Laboratory studies are performed to validate the efficacy in a laboratory according to criteria defined. These tests permit to validate for example a level of mortality during a given time, a knock down (KD) effect and if need be the palatability of the product.

Simulated-use tests are more linked to practical/field conditions and can, in some cases, be sufficient for demonstrating the efficacy. Simulated-use tests can include factors like ageing, weathering, UV, washing, etc. Example: For disinfecting products aimed at controlling bacteria on hard surfaces, it is sufficient to carry out a suspension test and a surface test in accordance with the relevant EN standards.

Field trials provide a good indication of how the product works in practice/under field conditions, to evaluate how the efficacy can be affected by a variety of factors (the weather, population density, natural fluctuation of the population over time etc.). The experimental setup is important in these tests. The results of the tests should be compared to the results achieved with a control object which has not been treated or with the situation prior to treatment: however, in some cases it is not possible to include a control sample in field trials.

Screening tests, laboratory studies and simulated/use tests must always include an untreated control without active substance (i.e. a negative control); it is preferred that this is the formulated product without active substance. However, providing it can be justified,

this can be, a control with only the solvent, e.g. water. There are few exceptions to this rule, such as the EN disinfection test, and all exceptions should be justified by the methodology.

Tests should preferably be carried out in accordance with standard protocols (e.g. CEN, ISO, OECD, ASTM, etc.). If standard protocols are not available or are not suitable for the field of use concerned, other methods may also be used on condition that the studies concerned have a sound scientific basis. Preferably, available standard methods should be modified to meet the actual application in such cases. Ideally, tests are carried out in accordance with Good Laboratory Practice (GLP) or similar quality assurance systems (ISO), although this is not mandatory for efficacy tests.

3.1.2 Test report

Some standard tests (e.g. EN tests) contain examples of appropriate reports, which should be used as a template. In all other cases the test report must contain the following elements:

- introduction
- materials and methods (e.g. tested product composition, conditions of the test temperature, humidity,)
- tested organisms
- results and raw data
- conclusion/discussion based on criteria defined in guidance

The introduction must indicate the goal of the test. When a standard test is used the name and/or number of the test should be stated. The section on materials and methods must provide a complete description of the test method. If an internationally recognised standard method is used, it is sufficient to provide a brief description of the test. The product used and the concentration of the active substance must be specified. If the name of the product tested is not the same as the product for which the application is being submitted (e.g. a name used outside the EU or an internal company code for the product), the complete composition of the product tested must be provided in a separate document. The test organisms used must correspond to the organisms against which the product is intended to be used, or they must be adequate representatives. For example, if a product is intended for use against bacteria in hospitals, it is not possible to test the product on all possible species of bacteria. Instead, four standard species of bacteria are usually tested. The conditions under which the negative control tests were carried out must also be described (e.g. treated with product not containing the active substance, not treated, or treated with water for example).

The materials and methods should be described well. In case of standard test protocols all the deviations should be indicated and justified.

The section on the results of the test must provide quantitative data. It is not sufficient to present only tables or figures in which the results have been processed. The raw data must also be included. In case of repetitions performed in the test, the results should also be subjected to a statistical analysis, when appropriate. At the end of the report, a conclusion must be presented. Sometimes, it is necessary to discuss and/or present further arguments for the conclusion. For field trials in particular, the results obtained in repeated tests may differ. If an explanation is provided for such differences in results, a test may possibly still be approved.

Example: In test 1, the product was "washed away by rainfall" and was therefore not effective, but tests 2 and 3 do demonstrate the efficacy. In such case the tests can be

accepted and a remark will be made on the SPC that the product should not be used when rain is expected within x hours, because this will influence the efficacy negatively.

When applying for authorisation all the efficacy tests should be summarised in the PAR. The PAR format includes a table. This table should be filled out in a way that it gives an overview of all the efficacy results. When the test is not a standard test a short description of the method should be included. The test column "test system/concentration applied/ exposure time" should include all the relevant information on the test, the test parameter (e.g. contact time, temperature, replicates) in way that it can be compared to the intended use. The results should be specified (e.g. x% mortality, lg reduction >x) and not just "test passed". In some cases it might be easier to summarise the results in the text instead of the table (e.g. field trials).

Below the table the tests should be discussed and an explanation should be given on how the test results demonstrated the efficacy of the product for the different uses under use conditions.

3.2 Resistance

The topic of resistance is discussed in the general part of the TNsG on Product Evaluation (Section 6). Information on resistance should be given for active substances and biocidal products. Additionally, in support of the review for each active substance, information on resistance is given in the Competent Authority Report (CAR) of this active substance.

Resistance will be assessed on the basis of expert judgement. This section of the guidance will be updated in the future in the light of experience gained in evaluation of resistance.

4. Active substance approval

4.1 Introduction

According to Article 4 of the BPR, an active substance must be approved if at least one biocidal product containing that active substance may be expected to meet the criteria laid down in point (b) of article 19(1), and more particularly for the context of this guidance the paragraph (i), which says "the biocidal product is sufficiently effective".

During the review of an active substance at the active substance approval stage, both the efficacy of the active substance and of the representative biocidal product are assessed in a relevant matrix. At this approval stage, it is the activity of the active substance which must be demonstrated, both in its own right and when formulated into a biocidal product.

Although a biocidal product containing the active substance is evaluated at the active substance approval stage, this part of the BPR process is concerned primarily with the efficacy of the active substance itself. The purpose of this section is to provide guidance for applicants and competent authorities on the principles for evaluation of efficacy at the active substance approval stage, and to help determine whether the information provided in an application for approval of an active substance is sufficient for inclusion of the substance in the Union list. For guidance on data requirement see Volume II Part A of ECHA's guidance under the BPR.

4.2 General principles

4.2.1 Intended use

When making an application for approval of an active substance, the applicant must clearly describe the uses for which the active substance is intended. This information is required to allow a proper evaluation of the efficacy to be carried out, and must include, for every product type separately:

- The purpose of the claim (e.g. prevent destruction of material by insect infestations, decrease risk of infection by bacterial contamination);
- The function of the active substance (e.g. bactericide, fungicide, rodenticide, insecticide);
- The (group of) target organism(s) to be controlled;
- The effects on representative target organism(s) (e.g. attracting, killing, inhibiting);
- Any products, organisms or objects to be protected.
- The likely concentration at which the active substance will be used in products and, where appropriate, in treated articles. This likely concentration should be demonstrated to be effective according to the requirements described in section 4.2.2.1.

In the application, the applicant may choose to provide information on all of the intended target organisms at the active substance approval stage, or a representative selection.

However, in order for approval of the active substance to be granted, efficacy must be demonstrated for at least one main target organism (or group of target organisms, e.g. bacteria). Use against additional target organisms may be applied for at the product authorisation stage.

For active substances used in treated articles, see section 4.5 and sub-sections 4.5.2 and 4.5.3.

4.2.2 Evaluation of efficacy

Efficacy of an active substance has to be demonstrated both in part A of the CAR (related to the intrinsic efficacy of the active substance) and in part B (where the active substance is incorporated in a formulated product). Evaluation of each part is described below.

4.2.2.1 Active substance efficacy (part A):

As the testing of an active substance is normally carried out using the technical active substance, or a simple dilution of the active substance in water or an appropriate matrix (so that the testing is carried out in the absence of other substances which may affect the efficacy), an extensive data package and evaluation is not required at this stage.

However, efficacy studies should be submitted on the active substance, and these data should be capable of demonstrating the innate activity of the active substance against representatives of the proposed target organisms at the concentration relevant for the risk assessment. For that purpose, innate activity of an active substance could be defined as the capacity of an active substance to provide a sufficient effect on one or several relevant target organisms, for the use considered.

The following minimum requirements should be fulfilled to demonstrate innate activity:

- For main group 1 (disinfectants: PT1, 2, 3, 4 and 5), innate activity is at least a "cidal" activity demonstrated in a suspension test and has to be demonstrated against one or more representative target organism(s) for the activity claimed (e.g. bactericide, yeasticide), preferably according to the CEN norms (phase 1 tests and phase 2 step 1 tests). Test organism(s) should be that or those specified in the respective norm. Phase 1 tests are sufficient for the active substance if a phase 2 step 1 test is available for the representative product. When only specific biostatic activity (e.g. bacteriostatic, fungistatic) is claimed, an appropriate method should be used.
- For main group 2 (preservatives: PT6, 7, 8, 9, 10, 11, 12 and 13), innate activity is generally a static activity demonstrated in challenge tests on several and relevant target organisms, in the relevant matrix. However, if curative effects are claimed, cidal activity is requested. To demonstrate efficacy against one target organism only could also be acceptable in the case of a strictly defined use relevant for the PT (e.g. the control of Legionella in cooling water in PT11). For PT8, CEN norms are available to support efficacy testing and give indications on representative target organisms to be tested. Growth in the untreated control is essential to show the validity of the test. If the claim is only for a curative effect, it is sufficient to show that the decline in the microbial population in the treated samples is statistically significantly more than in the untreated control samples.
- For main group 3 (pest control: PT14, 15, 16, 17, 18, 19 and 20), innate activity can be demonstrated for one target organism only (for instance, control of mice or control of bedbugs).
- For main group 4 (other biocidal products: PT21 and PT22), innate activity is generally supported on a group of organisms (algae, animals, bacteria) and examples of appropriate target organisms are available in the Efficacy guidance for PT21 and PT22.

When minimum requirements are not met this should be justified.

Generally, efficacy data are generated from laboratory tests, performed by the applicant. Nevertheless efficacy data from literature could also be acceptable if the application rate, target organisms, area of use and the identity of the active substance is described and are relevant. If cited literature is used to support a preserving effect it must also show that untreated test specimens supported growth. When curative effects are claimed the cited literature must demonstrate the efficacy of the active substance according to the requirements per PT. The use of cited literature should be agreed between the applicant and the CA on a case by case basis.

The level of efficacy demonstrated at this stage of the process need not be high, as an active substance in a simple solution may not be as effective as when it is used in a fully formulated product. For that reason an active substance should still be considered suitable for approval if the levels of efficacy demonstrated fulfil the minimum requirements above. In the case where the levels of efficacy of the active substance alone are lower than expected, efficacy tests performed with the representative product has to show a sufficient/basic efficacy, according to the requirements above. If both are insufficient, approval for the Union list should not be proposed.

If no efficacy tests with the active substance itself are available, but only tests with a formulation, a justification has to be given by the applicant regarding the possible influence of co-formulants on the efficacy. If the co-formulants used potentially have biocidal activity, it is essential to demonstrate that the efficacy is due to the active substance and not to the

co-formulants (e.g. a control should be performed with all co-formulants but without the active substance).

4.2.2.2 Product efficacy (part B):

Although approval for the Union list is primarily concerned with the active substance, efficacy data is also required for a representative product. Ideally efficacy data on an existing biocidal product should be submitted. If this is not possible data on a dummy product could be acceptable in order to demonstrate that the active substance is capable of producing an effect on the target organism and in a relevant matrix according to the proposed use, when included in a formulated product.

However, a detailed evaluation of the effectiveness of the product (including an evaluation of the proposed label claims) is not in all cases required at the active substance approval stage. This may for example be the case where no marketed product is available.

Nevertheless, the level of efficacy (e.g. the kind of activity "biocidal" or "biostatic") have to be consistent with the uses claimed and fulfil the minimum requirements mentioned in the active substance part (part A).

4.2.3 Overall evaluation for active substance approval

It is concluded that efficacy data are required on the active substance, to demonstrate on the one hand the innate activity of the substance (either the technical grade active substance or a dilution in water or a solvent) and on the other hand the efficacy of the representative product against one or more of the proposed target organisms. Efficacy should be demonstrated in accordance with the use(s) considered in the risk assessment. If for some justified reasons, the results of the biocidal product do not completely fulfil the requirements described above, this could still be acceptable as long as the results of the active substance are sufficient to demonstrate efficacy. The other way around, if the results of the active substance do not fulfil the requirements described above acceptable data of the biocidal product may be sufficient as long as it can be excluded that the co-formulants contribute to the efficacy of the product.

Where the levels of efficacy demonstrated are low enough to raise concerns by the evaluating Member State, the applicant should be asked to justify why the result should still be considered acceptable. Two specific reasons are discussed below: the use of 'dummy products' and the case of active substances not used alone but always in combination with other active substances.

4.2.4 Link to risk assessment

There is an essential link between efficacy testing and the risk assessment for human health and the environment at the active substance approval stage:

- Efficacy has to be proven for active substance concentrations used in the risk assessment
- Efficacy has to be sufficient for the use assessed in the risk assessment.

The information on efficacy is relevant in assessing the dose recommended for the use(s) applied for. The dose (or the "likely concentration(s) at which the active substance will be used" as stated in Annex II 6.4 of the BPR) is the starting point in the exposure assessment for human health and the environment.

4.3 Active substances which are not intended to be used in isolation

This section is developed to deal with active substances which are not intended to be used as the sole active substance in a product.

At the active substance approval stage, the following should be demonstrated:

- **in part A** (dedicated to the active substance), the innate activity of the active substance should be demonstrated against target organism(s) relevant for the field of use envisaged.

The evaluation should demonstrate that the active substance is capable of producing an effect on its own or when formulated into a very simple product. Due to the absence of the other active substance(s), the formulation may have only a limited, rather than broad based, spectrum of activity, or a lower level of efficacy.

Evaluation of the data will be done on a case by case basis.

Some examples where limited efficacy could be acceptable:

- for wood preservatives with fungicidal activity where different fungicides are active against different groups of target fungi and therefore two or more fungicides would be included in a product to produce the full spectrum of antifungal activity;
- for insecticides that are used in combination with other active substances to improve the insecticidal performance of the latter as they exert a synergistic effect;
- for insecticides used in combination with a co-formulants (e.g. booster) that is not itself an active substance;
- the active substance is used in combination with another active substance.

However, an appropriate argumentation is always required in order to justify situations with a more restricted level of efficacy. The minimum requirements in section 4.2 have always to be fulfilled.

- **in part B** (dedicated to the accompanying/representative product), the efficacy of a product where the active substance is formulated in combination with other (active) substances should be demonstrated against target organism(s) relevant for the field of use envisaged. Relevant efficacy tests should be used and structured to allow evaluation of the contribution of the active substance to the overall efficacy. This is particularly important if efficacy data have not been submitted in part A.

Efficacy data packages for formulations containing two or more active substances are not fully suitable for determining the activity contribution from the active substance under evaluation. For that reason great attention should be paid to justify the contribution of the active substance under evaluation to the total efficacy of the product. Information about the mode of action/function of the other active substances present in the product is also requested.

The submitted data should allow the definition of an effective concentration (i.e. the concentration of active substance at the efficient application rate of the product) that can be used for the risk assessment (specified per use). If in part B a formulation is introduced with additional co-active substances, this formulation will only be considered for efficacy testing and for setting a likely in-use concentration of the active substance, not used in isolation.

A statement should be added in the BPC opinion in order to stress that the active substance is intended to be used in combination with other active substances or synergists.

4.4 "Dummy products"

A "dummy product" is a product that is not fully formulated. It is not intended to be placed on the market.

In order to satisfy the requirement of the BPR, a dossier of an active substance for inclusion in the Union list (or in Annex I of active substances referred to in Article 25a of the BPR) may be accompanied by such a product as the associated biocidal product. To the extent possible, data from real products are nevertheless recommended.

While some dummy products may be very similar to a fully formulated product, others may be a very simple formulation that bears little resemblance to the product which will finally be placed on the market. The latter may be used where the applicant has limited experience in formulating products, for example by applicants who only manufacture active substances.

At the active substance approval stage, the following should be demonstrated:

The evaluation should demonstrate that the active substance under evaluation is capable of producing an effect when formulated into a very simple product (active substance alone or diluted in a solvent) and to define an application rate, which is consistent with the intended use(s) claimed by the applicant, and that can be used for the exposure assessment.

If a dummy product is used, a more restricted level of efficacy could be acceptable if an appropriate and detailed justification is given by the applicant. However, the minimum requirements mentioned in section 4.2 have always to be fulfilled.

4.5 Active substances used in treated materials and treated articles

Treated articles have been included into the biocides legislation on 1 September 2013 with the BPR (Biocidal Products Regulation). This requires different considerations and testing approaches as compared to the previous legislation, BPD.

Guidance on treated articles is further addressed in sections 5. 3 and 5.4.6.

4.5.1 Efficacy assessment for active substance approval

For biocidal products placed on the market in the EU, the authorisation requirements of the BPR apply, including testing efficacy. For treated articles imported into the EU, there is only the active substance approval stage to test efficacy. In this respect, it is particularly important to evaluate and assess use in treated articles at the active substance approval stage.

Where claims to treat articles are made for active substance or biocidal products, efficacy data to support these claims have to be submitted (see Annex II, Title 1, 6.6 and Annex III, Title 1, 6.6 and 6.7). If claims are made on active substance level, efficacy assessment of the use in treated articles has to be part of the active substance evaluation.

4.5.2 Efficacy assessment for active substances in specific PTs

For active substances notified for certain PTs it is obvious that they are mainly, or exclusively used, to treat articles/materials as for example for PTs 6, 7, 8, 9, 10 (Main group 2). Thus, efficacy testing with respect to use to treat articles/materials, is a natural part of the active substance evaluation. In such cases use concentrations and standard use

conditions for use in treated articles have to be taken into account in assessing efficacy. The biocidal function of the PTs within Main group 2 is usually protection of specific materials from biodeterioration, in some cases odour prevention. The state of the articles treated can be solid or liquid. The use conditions can be dry, humid or wet, which can be quite crucial for the release of the active substance out of the matrix. Thus, the representative product should show the claimed effect(s) in the range of uses and use conditions which are described and in the type of matrixes applied for. Use conditions like ageing, weathering or washing should be simulated as appropriate, to demonstrate the duration of the effect in relation to the life-span of the article treated.

Active substances notified for PTs 1-5 (Main group 1) are usually used in (liquid) biocidal products as for instance hand disinfection or surface disinfection products. These products are clearly considered biocidal products. But sometimes active substances belonging to PTs 2, 3 or 4 are incorporated into textiles and other solid materials; the protection of the material itself is not intended, but a new property is introduced to an article, intended to protect its user. For such claims, testing is particularly challenging and the specific conditions of use have to be considered when designing the efficacy testing. Please read more about how to design such tests in section 5.4.6. At active substance level, the representative product should show the claimed effect(s) in a range of uses and use conditions which are described and in the type of matrixes applied for. Particularly the wet state of the use conditions (dry, humid or wet) needs to be taken into account, as this is crucial for the release of the active substance out of the matrix and thus for the efficacy of the representative product. Furthermore, use conditions like ageing, weathering or washing should be simulated as appropriate, to demonstrate the duration of the effect in relation to the life-span of the article treated. Use conditions for which no efficacy of the representative product could be demonstrated must be excluded from the approval as appropriate.

Active substances belonging to PTs 18 and 19 and used to treat (solid) articles can have different purposes. The treatment can be intended to protect the material (for instance a carpet treated with an insecticide to prevent moth damage) or it can be intended to protect humans or animals against insects (for instance clothes treated with a repellent). Again, in the latter case it has to be carefully considered whether such a product fulfils the definition of a biocidal product and has to undergo an authorisation procedure. At the active substance approval stage, any claims made should be demonstrated with appropriate efficacy tests on the representative product, taking into account the specific conditions of use (e.g. regular washing for clothes) and the availability of the active substance to the target organisms, which can differ in different matrices.

5. Product authorisation

5.1 Evaluation of efficacy at product authorisation stage

The Product Authorisation stage is the point in the evaluation process where the efficacy of the biocidal product should be looked at for the full range of claims made. More test organisms or different uses can be relevant as compared to active substance approval. At this stage, it is not the properties of the active substance which are of interest, but instead the properties of the fully formulated product, which may contain more than one active substance.

Therefore, this is the stage at which a full evaluation of the efficacy of the formulated product should be carried out, and where the efficacy is evaluated in relation to the label claims made for the product. This evaluation should include all relevant target species (or representative species), the effects of using the product, the duration and speed of effect (including ageing and weathering if relevant), any claims for residual action, together with any other specific claims.

At biocidal product authorisation stage, the applicant must clearly describe the uses for which the product is intended when it is used under normal conditions, at the appropriate application rate and in accordance with the use instructions.

This information is required to allow a proper evaluation of the efficacy to be carried out, and must include, for every product type separately:

- The purpose of the biocide (e.g. prevent destruction of material by insect infestations, decrease of bacterial contamination on surfaces);
- The function of the product (e.g. bactericide, fungicide, rodenticide, insecticide);
- The organism(s) to be controlled;
- The effects on representative target organism(s) (e.g. attracting, killing, inhibiting);
- Any products, organisms or objects to be protected;
- The concentration at which the active substance will be used (the use concentrations for different targets should be stated for each use and method of application, if appropriate. Applicants should also indicate if the use concentrations should be different in different parts of EU);
- Description of the instructions of uses.

At the product authorisation stage, efficacy must be demonstrated against all claimed target organisms. Use against additional target organisms (i.e. which were not supported at the active substance approval stage) may be applied for at this stage.

For biocidal products used to treat articles, it is important to categorise possible wide ranges of uses into sets of similar materials and use-conditions. Please see sections 5.3, 5.4.2 and 5.5 for more details.

5.2 Product families

NOTE to the reader:

This section contains some information developed in accordance with CA-Nov14-Doc.5.8 that is now obsolete. This will be revised at the next update, but in the meantime, readers should use the information from this section in conjunction with the CA-July19-Doc4.2-Final - Note for Guidance implementing the concept of the biocidal product family[2] and BPC-37 - Harmonized approach to determine a worst-case (or a representative) test product to be taken into account for efficacy core assessment for a disinfectant BPF[3]

5.2.1 Background

A product family is a group of products with the same active substance(s) and similar use, but small differences in the formulation, which do not significantly reduce the efficacy of the products.[4]. When authorisation is requested for a product family efficacy should be demonstrated for the whole group but not necessarily of each product. A product family can be divided into different *meta*-SPC', and all products in the *meta*-SPC have the same hazard and precautionary statements. However, it is also possible that extra *meta*-SPC's should be added because of the efficacy assessment (e.g. some products in the family are not efficacious for some uses). It should thus be noted that the efficacy evaluation of the product family should be made in conjunction with the other parts of the evaluation (e.g. ENV, HH and phys-chem) and that an overall assessment of the division into *meta*-SPC's should be made taking all areas into account. This guidance is specifically aimed at an evaluation of differences in efficacy claim, which could lead to certain structures of the BPF and *meta*-SPC's. Therefore, some of the following examples could result in other structures of the *meta*-SPC's when environment, human health and phys-chem are taken into account.

5.2.2 Worst case testing

The BPF concept allows read-across of data between similar products within and across *meta*-SPCs. Efficacy tests must be performed on the product with the lowest concentration of the active substance, under the worst-case circumstances. The influence of the co-formulants on the efficacy should be taken into account. A justification should be given for the product and circumstances taken.

Tests and criteria for testing the efficacy of products in a family are the same as for single products. For the data requirements and test criteria, please see the specific sections per PT.

Applicants need to ensure that all products within a family have been supported, in terms of:

- target organisms;
- concentrations/application rates;
- contact time;
- influence of the co-formulants;
- application methods;
- field of use/use conditions;

[2] See CA-July19-Doc4.2rev2

[3] See BPC-37

[4] See Article 3 of the BPR for the full definition of a BPF.

- other label claims;
- formulations;
- any other relevant information.

Table 1: Example ready-to-use disinfectants with/without pre-cleaning*.

	Family A Concentration AS: 1-4%		
	***meta* SPC 1**		***meta* SPC 2**
	Product 1	**Product 2**	**Product 3**
concentration AS	1%	1%	4%
target organisms	bacteria yeasts	bacteria yeasts	Bacteria yeasts viruses
use conditions	apply after pre-cleaning	apply after pre-cleaning	apply without cleaning
Colour	1	2	1

NOTES to Table 1

In this example, one worst-case for efficacy cannot be identified. Product 1 should be tested against bacteria and yeasts under clean conditions (also supporting product 2), and product 3 should be tested against bacteria, yeasts viruses, under dirty conditions.

Since these are all ready-to-use products, and presuming that 1% is not efficacious against viruses, product 1 and 2 should be in a different *meta*-SPC than product 3 since they are not efficacious against viruses. The *meta*-SPC of products 1 and 2 will state as target organisms bacteria and yeasts and the *meta*-SPC of product 3 bacteria, yeasts and viruses.

***** In the examples, only the information given in the table is taken into account for the deviation in *meta*-SPC's, presuming that all other factors are the same for the different products or of no influence. In practice, other factors relating to the products will also need to be taken into account.

In some cases it is not possible to identify one worst-case scenario for a combination of products and use conditions: where such a single "worst-case" scenario at *meta*-SPC level cannot be identified, an assessment of the minimum efficacy levels that might be relevant for the uses covered by a *meta*-SPC has to be performed. For instance, the family contains products (1) and (2) with low active substance (AS) concentration which will be used as disinfectant under clean conditions and only for the control of bacteria and yeasts, while another product (3) with a higher concentration of AS is used under dirty conditions for the control of bacteria, yeasts, and viruses. Product (1) and (2) will not be sufficiently efficacious against viruses, so it cannot be used to demonstrate efficacy for all the uses. In this family, product (1) should be tested under clean conditions against bacteria and yeasts (and cover product (2)) and product (3) should be tested under dirty conditions against bacteria and yeasts and viruses (see Table 1: Example ready-to-use disinfectants with/without pre-cleaning*.Table 1). Tests done for a product in one *meta-SPC* can, where relevant, be used to support a claim for a similar product in a different *meta-SPC*, provided that variations in co-formulants have no influence on efficacy. Justification may need to be provided to allow read-across.

In some product families several combinations of products and uses should be tested, to demonstrate efficacy for all combinations of products and use conditions (see Tables 2, 3, and 4).

Table 2: Example concentrated disinfectants

	Family B Concentration AS: 10-40%		
	***meta* SPC** Product: 10-40% AS Dilute product to use concentration: bacteria: 1% AS fungi: 1% AS viruses: 4% AS		
	Product 1	**Product 2**	**Product 3**
concentration AS	10%	20%	40%
target organisms	bacteria fungi	bacteria fungi	Bacteria fungi viruses

NOTES to Table 2

In this example, all products are concentrates to be diluted before use. The applicant only claims efficacy against bacteria and fungi for products 1 and 2 and in addition viruses for product 3. Presuming all products only differ in the concentration active substance, testing can be done with either of the products at use concentration: product diluted to 1% active substance should be tested against bacteria and fungi, and product diluted to 4% active substance should be tested against viruses.

Since all concentrated products can be diluted to an efficacious concentration, when used according to the instructions on the *meta*-SPC, all products can be in one *meta*-SPC.

Table 3: Example surface disinfectants ready-to-use: more PT's

	Family C Concentration AS: 10%		
Option 1	***meta* SPC 1** Use #1: PT3, bacteria, fungi Use #2: PT4, bacteria, fungi, viruses		
Option 2	***meta* SPC 1** Use #1: PT3, bacteria, fungi		***meta* SPC 2** Use #2: PT4, bacteria, fungi, viruses
	Product 1	**Product 2**	**Product 3**
concentration AS	10%	10%	10%
target organisms	bacteria fungi	bacteria fungi	Bacteria fungi viruses
PT	PT3	PT3	PT4

NOTES to Table 3

In this example all products are ready-to-use and have the same use concentration, they only have a different use claim (i.e. same use in different PTs). It is presumed that the products only slightly differ in their composition and that it is demonstrated that this does not influence the efficacy. In this case, either of the products can be tested under worst-case conditions (justification should be given that PT3 soiling and temperature is the worst-case). A representative product should be tested against the specified bacteria and fungi required for PT3, and against the specified bacteria and viruses required for PT4. Since the fungi that have

to be tested for PT3 and PT4 are identical, one test performed under the worst-case conditions is sufficient. Since this *meta*-SPC can be split into 2 uses, one for PT3 and one for PT4, and all products are efficacious against all uses, it is possible to put all three products in one *meta*-SPC, (option 1). All possible products in this *meta*-SPC will be efficacious against use #1 and use #2. Efficacy against viruses in PT3 is not demonstrated, however, since this is not in one of the uses in the *meta*-SPC, this is acceptable. On the product label only the specified uses, combination of PT and target organisms can be claimed. However, an applicant might consider it easier to split the family into 2 *meta*-SPC's, one per PT (option 2).

Table 4: Example insecticide: take target organisms and application method into account.

	Family D Concentration AS: 1-4%		
	***meta* SPC 1** Conc. AS: 1%	***meta* SPC 2** Conc. AS: 1%	***meta* SPC 3** Conc. AS: 4%
	Product 1	**Product 2**	**Product 3**
concentration AS	1%	1%	4%
target organisms	Moth	moth and mosquitoes	Ants
application method	paper in wardrobe	electric device in wardrobe or room	bait box with sugar

NOTES to Table 4

In this example, one worst-case for efficacy testing cannot be identified and all products should be tested for all target organisms and uses.

All three products should be in different *meta*-SPC's because of the different application methods and organisms.

When a family contains more than one active substance it might not be sufficient to test the products to be authorised in a *meta*-SPC, in some cases it is necessary to test a 'dummy' product to cover all products in one *meta*-SPC (see Table 6). Alternatively, they could be authorised in separate *meta*-SPC.

5.2.3 Take formulation types and chemical composition into account

While the active substance is the most important constituent for the efficacy of a biocidal product, the effect of the formulation of the product on the efficacy must also be taken into account. Therefore, justification should be given for the product used in the test, taking into account the formulation. If the product contains more than one active substance, the combined effect between different active substances will be considered.

In the case of products having different formulation types (e.g. wettable powder and water dispersible granules for PT18), bridging studies with these products can be used to substantiate that the products are equivalent in terms of their efficacy. Bridging studies should involve worst-case circumstances (after appropriate justification).

Depending on the influence of the ingredients (chemical composition) on the efficacy either the product with the lowest concentration of all the ingredients should be tested or several products, together including the whole spectrum of the formulations, should be tested (see Table 5).

5.2.4 Allowing for the addition of new products in a family

In general, the (*meta*-)SPC(s) of a family will give a range for the concentration of the active substance(s) and co-formulants. After authorisation of the family it is possible to add new products to the family, as long as their composition falls into the range for the (*meta*-)SPC. For these new products, no evaluation will be done. Therefore, efficacy testing should be done in such a way that efficacy against all possible new products will be demonstrated.

For instance, in the example in Table 5, a new product with 70% active substance and the lowest concentration of both acids could be added. Efficacy of this product should be demonstrated, or the two products should be put into different *meta* SPCs. Another example is explained in Table 6.

Table 5: Example disinfectant: take formulation into account.

	Family E Concentration AS: 70-85% Concentration acid 1: 1-4% Concentration acid 2: 2-5%		
Option 1	***meta* SPC 1** Concentration AS: 70-75% Concentration acid 1: 1-4% Concentration acid 2: 2-5%		***meta* SPC 2**
Option 2	***meta* SPC 1**	***meta* SPC 2**	***meta* SPC 3**
	Product 1	**Product 2**	**Product 3**
target organisms	bacteria fungi	bacteria fungi	bacteria fungi virus
Active substance	70%	75%	85%
Acid 1	1%	4%	1%
Acid 2	5%	2%	5%

NOTES to Table 5:

In this example, both acids are pH regulators. It is presumed they are not considered active substances in this formulation (in some cases this should be demonstrated with tests), however, both acids might enhance the efficacy to some extent (i.e. formulation effect). Since it cannot be ruled out that there is a difference in effect between these two acids, this should be taken into account in the efficacy testing.

When products 1 and 2 are placed in one *meta*-SPC (option 1) it should be considered that it is possible to add a new product in this *meta*-SPC with 1% acid 1 and 2% acid 2. In that case it is not sufficient to test product 1 (with the lowest concentration AS), but a 'dummy' product should be tested, with 70% AS, 1% acid 1 and 2% acid 2.

To prevent testing with 'dummy' products, it might be easier to place products 1 and 2 in separate *meta*-SPC's, without a range for the acids (option 2). Also in that case, read-across between products 1 and 2 is not possible. Both products 1 and 2 should be tested, to rule out the effect of the formulation with different acid concentrations.

In all cases, product 3 should be tested against viruses, and put in a different *meta*-SPC (assuming 85% is necessary for viruses). The test with product 1 or the 'dummy' product can be used to demonstrate efficacy against bacteria and fungi for *meta*-SPC 2 (product 3).

5.2.5 Deviation in *meta* SPC's

When dividing a product family in *meta*-SPC's, it must be taken into account that all (possible new) products will be efficacious for all uses, target organisms, etc. Worst-case testing must make sure that all possible new products will be efficacious. Where needed/possible new *meta*-SPC's should be made for a different group of target organisms, a different use, different application method, etc.

This means for the example family in Table 4, that all products should be in a different *meta*-SPC.

In Table 1 products 1 and 2 should be separated from product 3, because these are not efficacious against viruses and therefore not against all target organisms in this *meta*-SPC.

However, in some cases it might be possible to not deviate in more *meta*-SPC's but give a good description in the *meta*-SPC, making sure that all products will be efficacious. For instance, in the examples in Tables 2 and 3, which are very similar to Table 1, the product with a virus claim can be in the same *meta*-SPC. This is acceptable because all possible products are efficacious when used according to the use description in the *meta* SPC, either because all products can be diluted to an efficacious dose, or by making separate use numbers. In these cases, some of the products in the *meta*-SPC have a limited claim (i.e. fewer organisms, fewer PT's).

When the different uses result in a too complicated *meta*-SPC, with several different use numbers, it is better to divide such a *meta* SPC in simpler *meta*-SPC's.

When dividing into meta-SPC's the applicant must make sure that the text in the *meta*-SPC's is unambiguous, and consider that no products can be added to the family that have not been supported in the efficacy testing (see Tables 3 and 4).

Table 6: Example anti-fouling product: Different ratios of two (or more) active substances.

	Family Concentration.AS 1: 5-10% Concentration.AS 2: 2-7%	
Option 1	***meta* SPC 1** Concentration.AS 1: 5-10% Concentration.AS 2: 2-7%	
Option 2	***meta* SPC 1** Conc. AS 1: 10% Conc. AS 2: 2%	***meta* SPC 2** Conc. AS 1: 5% Conc. AS 2: 7%
	Product 1 RTU	**Product 2 RTU**
target organisms	Macro fouling	Macro fouling
Active substance 1	10%	5%
Active substance 2	2%	7%

NOTES to Table 6:

In this example testing products 1 and 2 is not sufficient to cover the worst-case situation of this family. The worst-case would be a product 5% active substance 1 + 2% active substance 2. Assuming variation of co-formulants has no impact on efficacy, this 'dummy' product should be tested to demonstrate efficacy for this family when it consists of one *meta*-SPC (option 1). Alternatively, products 1 and 2 can be put into different *meta*-SPC (option 2), and efficacy tests using products 1 and 2 can be provided

5.2.6 Minimum concentration needed

Whilst ready-to-use products authorised on their own are evaluated on their merits and not in comparison to other products, this is not the case in a product family. Since all products are presented at the same time comparison can be made. The BPR Annex VI art. 77 of the common principles state: the recommended dose is the minimum necessary to achieve the desired effect.

For historical reasons, it is possible that products on the market in one EU country contain a higher concentration of AS than another product with the same intended use in another country. When this is the case the applicant should request authorisation for the products with the lowest concentration of AS or give a good justification why it is relevant to have different formulations.

It should be considered that there may be other products on the market which contains a lower concentration of AS and is efficacious for the same intended use.

5.3 Treated articles

NOTE to the reader:
This section concerns treated articles and should be read in conjunction with the CA Note for Guidance "Frequently asked questions on treated articles", CA-Sept13-Doc.5.1.e, Revision 1 December 2014 [5].

Article 3 Definitions

1. For the purposes of this Regulation, the following definitions shall apply:

(a) 'biocidal product' means

- any substance or mixture, in the form in which it is supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action,

- any substance or mixture, generated from substances or mixtures which do not themselves fall under the first indent, to be used with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action.

A treated article that has a primary biocidal function shall be considered a biocidal product.

(l) 'treated article' means any substance, mixture or article which has been treated with, or intentionally incorporates, one or more biocidal products.

A treated article according to Article 3(1)(l) of the BPR is any substance, mixture or article which has been treated with or intentionally incorporates one or more biocidal products. A biocidal product, in contrast, is any substance or mixture with a biocidal function. Pursuant to Article 3(1)(a) a treated article with a primary biocidal function is considered a biocidal product.

Liquids fulfil the substance or mixture definition. Consequently, liquids may only be considered as treated articles if they do not intend to control any harmful organism. In contrast, solid treated articles are defined by their shape and function rather than by their

[5] CA-Sept13-Doc.5.1.e.Rev1

chemical composition. Thus, solid treated articles fulfil the definition of a biocidal product if they have a primary biocidal function.

The term "primary biocidal function" is not further defined in the BPR, but in the CA document, it is described as "a biocidal function of the first rank, importance, or value compared to other functions of the treated article".

A biocidal product, in contrast, is any substance or mixture with a biocidal function. Consequently, efficacy testing and assessment is not principally different for biocidal products and treated articles. Both categories can take different forms (liquid, solid) and can concern different materials. In both cases, efficacy has to be shown for normal conditions of use and against an untreated control. The untreated control should demonstrate the problem which is to be solved by the biocidal treatment.

Thus, considering the different product types for PTs 1-4, the following examples would be considered as biocidal products and not treated articles. For PT 1 or 3, disinfecting wipes would be regarded as biocidal products[6]. For PT2, paints and coatings intended to prevent microbial settlement and growth in order to provide a hygienic environment would likewise be regarded as biocidal products[7]. Other PT 2 applications that could fall under either category, depending on their primary function could include for instance textiles, tissues, masks, or other articles or materials in which a biocidal product has been incorporated with the purpose of adding disinfecting properties to these articles and materials. For PT 4, examples are materials or articles which come into contact with food or feed and are treated with or incorporate a biocide; whether such articles are to be regarded as biocidal products again depends on their primary function. PT 5 applications are usually biocidal products. Further product examples are given in Appendix 1 of the CA document.

There are some exemptions in the definition given in Art. 3(1)(a): Articles such as paper or carton, where the pulp has been treated with a biocide during manufacture, and where the biocide is not intended to have a function in the final good are not considered treated articles. Another example are articles with print on it or with glue holding it together which have been treated with an in-can preservative. However, the preservative doesn't have any function in the final article as soon as the ink or adhesive is applied and dried. In contrast, an article like a table made of a composite material with wooden legs painted with a film preservative containing coating, is considered a treated article, as the coating still has a biocidal function in the final article.

Generally, there is no difference in efficacy testing of treated articles or biocidal products in a liquid matrix. For instance, wet state preservatives (PT 6) or a hand disinfectant (PT 1) are usually both tested in a liquid matrix, the first matrix is a treated article, the latter is a biocidal product; only the performance standards are different in these examples. Specific requirements apply, however, when the efficacy of solid material or articles has to be tested. A test under practical conditions of use (step 3 test) is mandatory. In contrast to preserving claims, where standard materials under certain standard conditions of use can be tested, testing for disinfecting claims has to be specific for every single article. For these types of claims, the specific conditions of use are to be considered when designing the efficacy testing; for example, a polymer coating used for a hospital bedside cabinet has to be tested for the specific contaminating situation of a hospital bedside cabinet, including cleaning schemes and soiling situation; efficacy has to be shown compared to an untreated bedside

[6] See CA-Sept13-Doc.5.1.e,Rev1 Appendix 1

[7] See CA CA-Sept13-Doc.5.1.e,Rev1 Question 8

cabinet. Bactericidal effects have to take effect very quickly to show an advantage compared to an untreated cabinet, where droplets of blood or saliva will dry out quickly and not either be contaminating any more. Please read more about how to design such tests in Section5.3.

Specific requirements apply, however, when the efficacy of biocides in solid material or articles has to be tested. Treated articles with claims to protect humans or animals fall under this category. In these cases, use conditions, most importantly humidity, have to be specified. Materials can be used in articles with a wide range of use conditions, and these have an effect on efficacy. For example, for a polymer article permanently exposed to water the conditions for bacterial growth are much more favourable, and different requirements apply as compared to a polymer article which is generally dry and is only exposed to occasional splashes or to the humidity which comes from touching it. But more importantly, humidity has an effect on the availability of the active substance, because it has to be released out of the matrix somehow. Another example are clothes treated with repellents; also in this case use-conditions do influence efficacy. Wearing and tearing and washing have to be taken into account to assess the efficacy. Complete protection time needs to be defined in terms of the life-cycle of the treated clothes.

Treated articles, if not biocidal products, do not require efficacy assessment under the BPR. However, active substances and biocidal products incorporated into treated articles may require assessment of their efficacy in treated articles as part of the active substance approval and biocidal product authorisation processes (if such uses are applied for).

Consequently, if efficacy is demonstrated for a certain set of use conditions, this cannot generally be transferred to another set of use conditions. The possible limits of the use conditions have to be reflected in the approval/authorisation decision. In the following, guidance is given for the testing of (solid) materials with claims to protect humans or animals.

There are two OECD test methods available:

- Guidance Document on the Evaluation of the Efficacy of Antimicrobial Treated Articles with Claims for External Effects (OECD Series on Biocides No. 1);
- Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials (OECD Series on Testing and Assessment No. 202 and Series on Biocides No. 8).

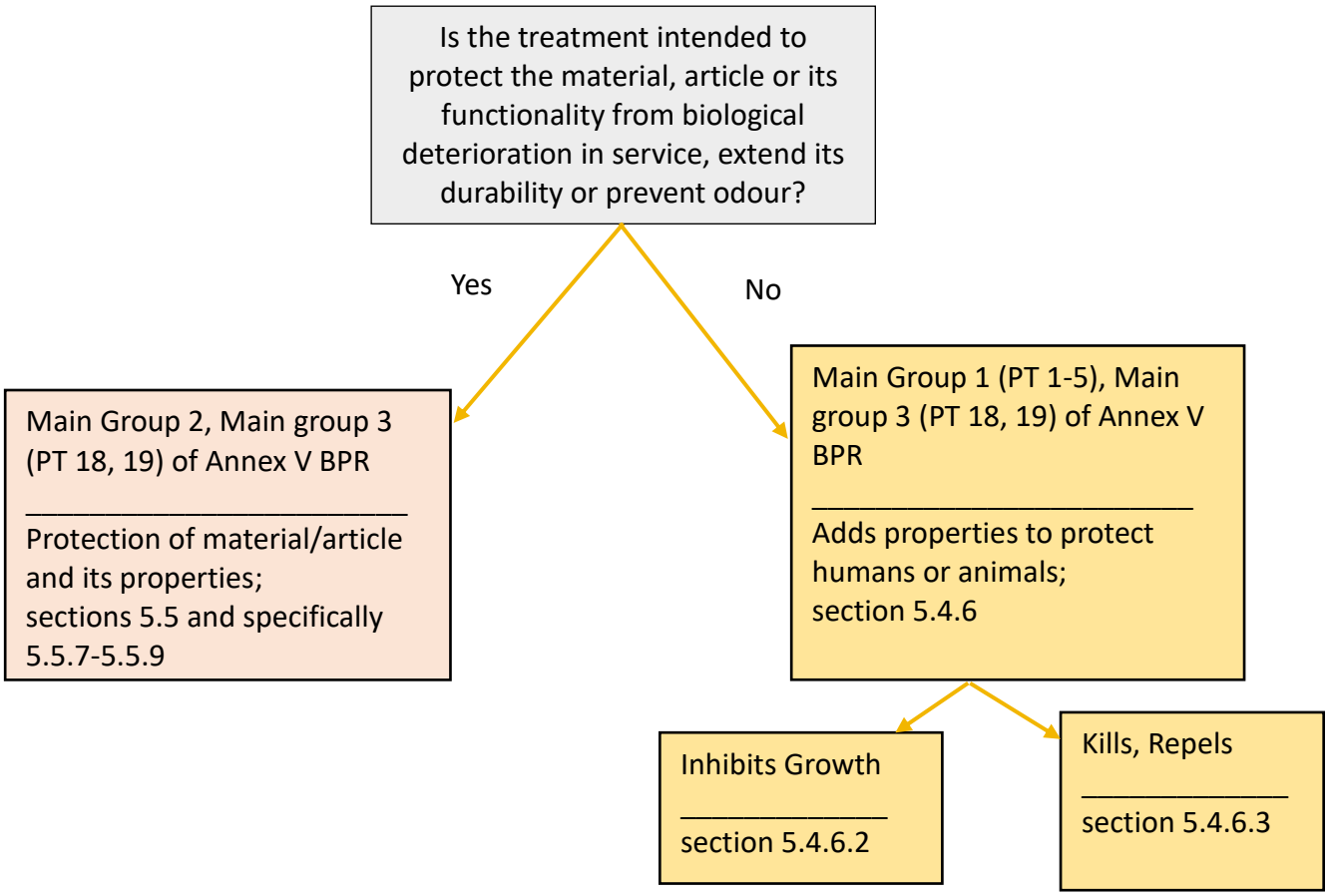
5.3.1 The basic distinction between material protection and protection of humans or animals

When biocides are incorporated into materials or used in the production of treated articles they are applied with two purposes:

- To protect the materials used in the article or the properties of the article in service. The target organisms have detrimental or other undesirable effects (e.g. biodegradation, discolouration, odour formation) on the material or article.
- To protect humans or animals from the unwanted effects of organisms. The treatment is directed towards target organisms which have no adverse effect on the item/material treated.

The following scheme gives an overview and decision help:

Figure 1: Decision scheme to distinguish between claims for material protection and claims for protection of humans and animals



Guidance for the testing of biocidal products with a claim to protect humans or animals is given in section 5.4.6. Guidance for material protection is given in section 5.5.

5.4 Disinfectants (Main group 1)

5.4.0 General

5.4.0.1 Introduction

This guidance describes the nature and extent of data which should be available to support the label claims for biocidal products within the Main Group 1: Disinfectants. This group covers 5 product types as described in Annex V of the BPR:

MAIN GROUP 1: Disinfectants

These product-types exclude cleaning products that are not intended to have a biocidal effect, including washing liquids, powders and similar products.

Product type 1: Human hygiene
Products in this group are biocidal products used for human hygiene purposes, applied on or in contact with human skin or scalps for the primary purpose of disinfecting the skin or scalp.

Product type 2: Disinfectants and algaecides not intended for direct application to humans or animals
Products used for the disinfection of surfaces, materials, equipment and furniture which are not used for direct contact with food or feeding stuff.

Usage areas include, *inter alia,* swimming pools, aquariums, bathing and other waters; air-conditioning systems; and walls and floors in private, public, and industrial areas; and in other areas for professional activities.

Products used for disinfection of air[8], water not used for human or animal consumption, chemical toilets, wastewater, hospital waste and soil.

Products used as algaecides for treatment of swimming pools, aquariums and other waters and for remedial treatment of construction materials.

Products used to be incorporated in textiles, tissues, masks, paints and other articles or materials with the purpose of producing treated articles with disinfecting properties.

Product type 3: Veterinary hygiene
Products used for veterinary hygiene purposes such as disinfectants, disinfecting soaps, oral or corporal hygiene products or with anti-microbial function.

Products used to disinfect the materials and surfaces associated with the housing or transportation of animals.

Product type 4: Food and feed area
Products used for the disinfection of equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption of food or feed (including drinking water) for humans and animals.

[8] This is taken to mean the disinfection of air itself. Disinfectants sprayed or vaporised into the air (e.g. room disinfection by vaporised biocide) are normally for the purpose of disinfecting surfaces and not the air itself. Disinfectants for air conditioning systems disinfect the surfaces or liquids in these systems, not the air coming out of them.

Product type 5: Drinking water
Products used for the disinfection of drinking water for both humans and animals.

Products in this main group are meant for the control of micro-organisms, such as bacteria (including vegetative cells, spores and mycobacteria), fungi (including moulds and yeasts), and viruses (including bacteriophages), algae and protozoa. Control may be carried out on inanimate surfaces or skin or in liquids. Note that the term "disinfectant" used for main group 1 should be read as a generic term and not according to the definition in the glossary of terms. This means that next to disinfectants it can also include products with biostatic activity.

The most important fields of use include medical, veterinary, food, feed and drinking water sectors. Applications in public, commercial and industrial areas, where the application is to inanimate surfaces without direct contact with food, are included in Product type 2. If contact between disinfected inanimate surfaces and food is possible (e.g. food industry, private and restaurant kitchens), applications are included in Product type 4.

Disinfectants for medical instruments and medical equipment that are considered medical devices are covered under the Medical Device Directive 93/42/EEC (see 3.9.1 for more information). More borderline cases with other Directives or Regulations are noted elsewhere in this Guidance Document and are defined or described in other legislation or guidance.

Cleaning products which are not intended as biocides, including liquid detergents, washing powders, etc. are excluded from these product types and thus this guidance is not applicable (Annex V of BPR).

Treated articles with claimed disinfecting properties or function can also fall within PTs 1 to 5: when such articles have a primary biocidal function, they are considered biocidal products (see Competent Authority (CA) document [9]). These articles can include a wide variety of goods, with different applications, matrices, etc. This guidance deals mainly with efficacy testing of (liquid) biocidal products; the methodology for testing (solid) treated articles can be quite different. See section 5.4.4.3 of this Guidance for details of available guidance.

A "Glossary of Terms" is at the beginning of the document.

5.4.0.2 Dossier requirements

The following aspects are relevant for the evaluation of the efficacy of biocidal products within PT1-5:

1. The label claim and instructions for use
2. Efficacy data of the product
3. The possible occurrence of resistance, cross-resistance or tolerance.

5.4.0.3 Label claim

For each product, clear label claims should be provided. When the label itself cannot contain all the necessary information, any accompanying leaflet should also be considered. To simplify the text only the term "label claim" will be used below. The information on the label has to be in line with the SPC.

The types of efficacy claims made for a disinfectant/ biocidal product depend upon, among other things, the types of micro-organisms the disinfectant targets (e.g. fungal spores, yeasts, mycobacteria, bacteria or bacterial spores) and the disinfectant's intended use (e.g.

[9] See CA-Sept13-Doc.5.1.e (Rev1)

in hospitals, in contact with food, in animal houses, in homes). Label claims and recommendations for use, including concentration and contact time, must be supported by the results of bactericidal, fungicidal, etc. tests appropriate to the area of application, which are normally performed on the basis of the specific standards. Complete instructions for use are an integral part of the label.

The information on the product label should fully correspond with the uses pre-defined at the authorisation stage and reflected in the corresponding version of the SPC[10]. Applicants must indicate clearly on the product's label the spectrum of antimicrobial activity claimed.

Examples of the common fields of applications are presented in the claims matrices which are a set of tables linked to this guidance document (see Appendix 1 for more information). The Claim Matrices are not intended to be exhaustive, but the majority of uses are included.

5.4.0.3.1 Target Organisms

The target organisms for which claims are made should be specified on the product label.

As the claimed antimicrobial efficacy for disinfectant products will encompass a large spectrum of potential target organisms, it is not necessary or indeed feasible to include all possible micro-organisms in an efficacy test designed to support a label claim. Instead, the types of target organism the product is intended for are mentioned, for example, fungal spores, yeasts, viruses, algae, protozoa, (myco)bacteria or bacterial spores.

Specific species are mentioned on the label where they are the only or most relevant organisms, or where they have different susceptibility to biocides than the rest of the group. For instance, mycobacteria are less susceptible than other bacteria and it is only relevant to control them in certain situations such as tuberculosis wards.

In general, it is not possible to claim against specific single species without claiming (and demonstrating) efficacy against the group of organisms (e.g. no claim against *Mycobacterium tuberculosis* without also making a general bactericidal claim, no claims against HIV without a general claim against enveloped viruses). However, there are some cases in which it can be justified that a claim only for a single or a small number of species is made (such as bacteriophages in the milk industry, or fungi *Aspergillus fumigatus* in poultry housing).

Claims against specific organisms or groups of organisms should not be made if they imply a false impression of the superiority of a product; for example, a claim against MRSA should not be made for a bactericidal product, because MRSA does not present a specific challenge for disinfectants.

Standard test methods normally specify one or more representative species that should be tested per group of organisms for which the claim is made. For instance, a bactericidal product should be tested on gram-positive and gram-negative bacteria, a fungicidal product should be tested on yeasts and fungal spores. The species used are representative species that take into account their relevance to practical use, susceptibility for disinfectants and adequacy for laboratory testing.

The test organisms and strains which should be used are normally stated in standard efficacy test methods, i.e. according to EN 14885 or OECD-guidance.

[10] Details on how to fill out the SPC are available in the ECHA Technical Guide and SPC Editor.

When it is not possible to use standard test methods for efficacy testing and other tests are used instead, the test organisms listed in Appendix 3 should be employed. If test organisms other than those listed in Appendix 3 are used, their relevance should be justified.

Wherever possible strains should be selected from international collections (their genetic stability should be checked regularly). The preservation procedures must be clearly described (EN 12353).

Other test organisms, in addition to those specified in the test standards, can also be tested. When efficacy against specific additional species is claimed, efficacy tests with those species should also be performed. In general, claims should not be made against the specific reference species used in a standard test as this can give a misleading impression that the product shows activity beyond that covered by the general (e.g. bactericidal, fungicidal) claim.

Mentioning specific organisms on the label is still a subject of discussion between the Member States. The above sections reflect the position at the time that this guidance is written.

For some areas of use, there are minimum requirements for the groups of organisms for which efficacy should be demonstrated. For instance, for products used for animal transport vehicles efficacy against bacteria, yeasts and viruses should be demonstrated. For these products, it is obligatory to test all required organisms. Per section, a sub-section on test organisms provides information on the minimum requirements for that use.

5.4.0.3.2 Areas of Use

Disinfectants are used almost everywhere that people want to "eliminate" or inhibit (for static products) micro-organisms. They are used to kill or irreversibly inactivate or inhibit bacteria, fungi and viruses on animate and inanimate surfaces and matrices, in hospitals, households, schools, restaurants, offices, swimming pools, kitchens, bathrooms, dairy farms, on medical and dental equipment, eating utensils and at many other locations.

In some cases, biostatic products are used which only inhibit micro-organisms (see section 5.4.0.5.3 of this guidance).

Applicants should clearly indicate the intended areas of use for the product on the label, for example, areas of use could include (not exhaustive):

- Hospital and other medical areas;
- Domestic use;
- Institutional use (offices, schools, etc.);
- Industrial applications (e.g. food, cosmetic, pharmaceutical industry, etc.);
- Restaurants and large-scale/canteen kitchens;
- Veterinary areas (animal housing, animal healthcare, teat or hoof disinfection, etc.);
- Recreational areas.

5.4.0.3.3 Sites of Application

In addition to the types of efficacy claimed, e.g. bactericidal, fungicidal, tuberculocidal, and the intended area of use, the applicant must specify the use patterns for which the disinfectant is recommended on the label.

Broad examples of use patterns (not exhaustive) could include areas such as:

- Use on intact skin;

- Use in hospitals, operating theatres, isolation wards, etc.;
- Use in food manufacturing, retailing, processing areas, etc.;
- Use in animal housing and equipment (e.g. pigs, sheep, poultry, etc.);
- Use on work surfaces, cutting boards, etc.;
- Use on fabrics or textiles;
- Use on toilets, bathrooms, sinks, etc.;
- Use against micro-organisms associated with human or animal waste;
- Use in air conditioning systems;
- Use in swimming pools, spas, aquariums and bathing waters;
- Use in tanks, pipelines, equipment soak or bottle wash.

5.4.0.3.4 Directions for use (Methods of application)

The label claim must specify the application method of the product. For disinfectants, there is a broad range of application methods (e.g. wiping, aerosol, spraying). The in-use concentration of the solution and the contact time, which are essential for safe and effective use, should be described on the label. Any other directions for use should also be specified, such as whether the surface should be cleaned first, and claims regarding the number of times a prepared use solution can be used (or re-used) before a fresh solution must be prepared.

The application method can have a strong influence on the efficacy of a product, therefore the testing of a product should be appropriate for the application method. If specific equipment is used for the application of the product (e.g. vaporisers) this should be taken into account when testing the product for efficacy. Equipment used in laboratory tests or small scale tests may (of necessity) be different from that employed in practice. This is especially the case when biocidal active substances are generated *in situ* using large scale equipment, such as electrolysis. In cases where small-scale tests cannot be extrapolated to actual use conditions a large-scale test with the equipment should be done.

5.4.0.3.5 Other interfering parameters

Any other circumstances that can influence the efficacy of a product should be mentioned on the label (e.g. temperature or pH requirements). For example, when a surface should be cleaned before applying the biocide and a no rinsing step is involved, or that alkaline cleaning fluid should not be used with acidic biocides, and *vice versa*.

5.4.0.4 Efficacy testing

For efficacy testing of disinfectants in general only quantitative tests methods should be used.

5.4.0.4.1 Tiered approach

For efficacy testing of disinfectants a tiered approach is recommended. The following tiers can be distinguished (in accordance with EN 14885):

- Phase 1 tests are quantitative suspension tests to establish that a product (or an active substance) has bactericidal, fungicidal, etc. activity without regard to specific conditions of intended use. Phase 1 tests cannot be used for any product claim.
- Phase 2 comprises two steps:

  - Phase 2, step 1 tests are quantitative suspension tests to establish that a product has bactericidal, fungicidal, virucidal, etc. activity, simulating practical conditions appropriate to its intended use.
  - Phase 2, step 2 tests are quantitative laboratory tests, often using carriers or living tissues with dried-on micro-organisms, simulating practical conditions to establish that the product has bactericidal, fungicidal, virucidal, etc. activity.
- Phase 3 tests are field trials under practical conditions.

Phase 1
Phase 1 tests are laboratory suspension tests to establish the basic activity of the product or active substance. These tests may be used during the development of the product, but are not accepted for product authorisation. However, a phase 1 test can be used to demonstrate that a co-formulant does not have any biocidal activity in the product.

Phase 2, step 1
Phase 2, step 1 tests are laboratory suspension tests in which the ultimate purpose is to establish at what concentrations the product meets specified requirements under "in-use" conditions. In these tests, in-use conditions (e.g. temperature, contact time, interfering substances) are considered in the test method.

Various laboratory methods have been developed for biocide activity testing. Although these experiments differ in their design and experimental detail, they are all based on the principle of adding a test inoculum to the disinfectant (or vice versa) and taking samples at specified times. The biocide in each sample is then neutralised and the survival of the organisms assessed. In practice, the methods can be classified into two groups, according to how the endpoint of the test is determined:

Quantitative tests
Samples of untreated and biocide-treated cells are plated on nutrient medium after neutralisation. After incubation, the number of colony forming units is determined and the lg_{10} reduction in viable counts is determined.

Capacity tests
The biocide is challenged successively with the test organism at defined time intervals. This type of test can be used for instance for swimming pools and toilet disinfectants which are challenged by new bacteria periodically. Following each inoculation, samples are taken, and after a suitable contact period has elapsed, the biocide is neutralised and the sample incubated in a suitable growth medium to determine the surviving micro-organisms. The result is expressed as the amount of the accumulated inoculum that was required to produce the "failure".

Phase 2, step 2
Phase 2, step 2 tests are simulated-use or practical tests, performed under rigorous conditions within the laboratory, which mimic real-life conditions, for instance by pre-drying the micro-organisms onto surfaces. These tests are used in a second testing stage. After measuring the time-concentration relationship of the disinfectant in an in-vitro test (phase 2, step 1), these practical tests are performed to verify that the proposed use dilution is likely to be adequate in real-life conditions. For several uses standardised, simulated-use tests exist (surface disinfection, hand wash or rub, instrument disinfection) but there are no standard tests available for many others.

Longer-lasting activity is claimed for some products. When these products are applied to surfaces, it is common that they will not be completely removed or rinsed off after application. This might lead to longer-lasting activity of the biocide on the surface. Likewise,

some products are used for maintenance via continued release of low levels of biocidal product. Both effects can be determined by appropriate efficacy tests.

Phase 3 Field or in-use tests
In-use testing involves the antimicrobial evaluation of the product under actual conditions of use on specified surfaces or materials in a specified environment. As with standard and non-standard laboratory methods, representative organisms or actual organisms of concern may be used.
Validated methodologies for these types of tests are currently not available, although some are in development.

The practical use conditions under which a product can be used can be very variable and are therefore difficult to standardise. Field trials, although not standardised, can however give valuable additional information on the efficacy of the product, provided that the studies are scientifically robust, well reported and provide a clear answer to the question. In these types of tests, a control treatment without biocide should be included. Where this is not possible, efficacy should be judged on a comparison of the situation before and after application.

Until validated standards are prepared, the responsibility for determining the acceptability of data derived from field trials in support of the claim will lie with the CA, taking into account the guidance given in EN 14885.

5.4.0.4.2 Standard test methods

Ideally, data should be generated using internationally or nationally recognised testing methods (CEN, OECD, ISO, etc.). Several international standard test methods currently exist for disinfectant products. Recommended standard tests are presented in Appendices 2 and referenced in Appendix 4 to this guidance document.

If there are no guidelines available for the specific use of a product, or guidelines are not suitable, the applicant may use other methods (such as intra-company Standard Operating Procedures), where the studies are scientifically robust, well reported and provide a clear answer to the question. In addition, the test methods used, together with the test conditions, should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with modifications to make the guideline more suitable for the specific product or use conditions, is also possible. EN 14485 provides guidance on modification of standards (EN 14485, section 4.2 version 2014).

At the time of publication of this guidance document, a broad range of CEN methods are available. OECD has several phase 2/step 2 test methods developed for the efficacy testing of disinfectants to be used on hard surfaces which have been published as Guidance Documents. Available tests are presented in Appendix 2 and referenced in Appendix 4. The use of CEN test methods is highly recommended, where these are available and relevant. However, it should be noted that although this Guidance is mainly based on EN standards, there are some cases where there are discrepancies compared to the EN tests. In such cases, the ECHA Guidance should be followed as the leading guidance. OECD test methods may be used if, for example, no CEN standard is available.

These methods, described below, typically give a standard set of test parameters, test organisms and pass criteria. Where specific conditions apply for a field of use, such as high/low level soiling, high/low temperatures, relevant contact times, etc. these conditions should be included in the efficacy tests.

CEN Standard Test Methods
A Technical Committee (TC 216) was established in the European Committee for Standardisation (CEN), to produce harmonised European methods for testing the activity of

disinfectants used in medical, veterinary, food, industrial, domestic and institutional areas. The standards are based on suspension tests (phase 1 and phase 2, step 1) and some simulated-use tests like surface tests (phase 2, step 2).

European standard EN 14885 gives information on the application and interpretation of European Standards for the testing of chemical disinfectants within product types 1, 2, 3 and 4 of the BPR.

This document outlines the various standards currently available and provides guidance as to the choice of available standards that may be used to demonstrate the effectiveness of disinfectants, in particular, situations (such as medical, veterinary and food hygiene) and on the interpretation of results from such tests in making and supporting efficacy claims.

In EN 14885 products intended for domestic use are grouped with products for use in food and industrial areas, and therefore the tests specified are not always relevant to domestic areas. For instance, the virus test EN 13610 only tests against bacteriophages. In these cases, the test from the medical area should be used where relevant. In cases where no test method is available for one area of use (e.g. sporicidal test in the medical area), a test from another area can be used instead, provided that the test parameters (soiling, temperature, etc.) are adapted to the intended use area (for further guidance on the adaption of tests see EN 14885.

The application of disinfectants to water systems such as swimming pools, spas, and drinking water is not addressed in EN 14885. For the evaluation of activity against *Legionella* in aqueous systems (water used in cooling towers and water for general purposes, like spas, pools, showers and other uses) a quantitative suspension test is available (EN 13623).

EN 14885 includes guidance on how a phase 3 field trial should be conducted. This guidance is intended to advise on the factors to be taken into account and controlled when performing a field trial.

The use of CEN test methods is highly recommended, provided that the methods are applicable for the use of a product. In some cases, the method can be adapted (other contact times, soiling, etc.) to fit the use conditions. Any deviation from a standard must be clearly described and a justification for any deviations provided.

OECD standard test methods

The OECD publishes practical test methods (comparable to phase 2, step 2 tests (1.4.1.3) or phase 3 (1.4.1.4)) for testing the efficacy of disinfectants on non-porous surfaces within the "Series on Testing and Assessment" or the "Series on Biocides", respectively. Currently, all available methods have been issued as OECD Guidance Documents. Guidance Documents are, however, not covered by the Mutual Acceptance of Data (MAD) principle and are advisory in nature. Further developed OECD Test Guidelines might become available in the future. As European Standards are not available for all types of applications yet, the use of OECD methods is recommended provided that the methods are appropriately reflecting the use of a product. Again, the methods can be adapted (other contact time, soiling, etc.) to better fit the use conditions, provided that any deviations from the standard are clearly described and justified.

Please note that in the OECD Guidance Documents on disinfectants, the volume of disinfectant solution added to the surface is very high compared to what is normally done in practice. This test protocol can only be used for uses where the volume of disinfectant solution per surface area is similar to the intended use (e.g. flooding).

Other standard test methods

While CEN standards and, in case no CEN standard is available, OECD methods are highly recommended, there are circumstances in which these tests cannot be applied, i.e. they are not available, or relevant to a particular product or use pattern. In those cases, other test methods can be used.

Other test methods, for example, VAH (former DGHM), DVG, AFNOR, US-EPA, AOAC or ASTM methods, are available and might be used when no international standard is available for a specific application. Where these methods lack predefined test parameters, target organisms or pass criteria, the applicant has to provide evidence why the chosen parameters are appropriate for the intended application.

Where no standard tests are available, suitable test protocols may be designed (and justified) by the applicant, but these should be discussed with and agreed upon by the CA before testing takes place.

5.4.0.4.3 Data requirements

Label claims and recommendations must be supported by the results of tests appropriate to the area of application.

In each test, the composition of the product to be tested should be clearly described, including the identity and function of the active substances specifying quality and quantity in the formulation. In addition, because the co-formulants can affect the efficacy if the product, they must also be clearly described including identity and function. Alternatively, the formulation can be identified by a retrievable reference name or number. In such cases (i.e. it may only state a code for the product for the purposes of confidentiality), the composition of the tested product should be provided separately. As the formulation may affect the efficacy of the product, the composition of the product tested should be the same as the product under consideration. If not, justifications should be provided for any differences, and these will be assessed on a case-by-case basis.

As phase 1 tests do not take practical use conditions into account, they are not considered acceptable to support claims during product authorisation. In general, phase 1 tests are used during the development of the product, for the inclusion of active substances on the "Union list of approved substances" under the BPR or to prove that a co-formulant has no biocidal activity.

In general, at least phase 2, step 1 and step 2 tests are required to support label claims during product authorisation. The phase 2, step 1 test will provide basic information on the efficacy of the product (in a standard test), while phase 2, step 2 tests investigate the effects of more in-use factors (such as drying of target organisms). The combination of phase 2, step 1 and step 2 tests will generally provide a robust data package to demonstrate the efficacy of a product. Deviations from the tiered approach should be justified.

In some cases, for example, when disinfection is done in suspension under real use conditions (because the target organisms are suspended in a liquid already or will be suspended during the process due to mechanical action, for example, in CIP), a phase 2, step 1 test is sufficient on its own, as this already simulates practical conditions.

In other cases a phase 2, step 2 test may be replaced by a phase 3 test where a phase 2, step 2 test is not appropriate. In general, a phase 3 test will be done in combination with a standard phase 2, step 1 test, as phase 3 tests are often variable.

Where in-use conditions cannot be simulated, phase 3 tests are required (e.g. drinking water disinfection with ionisation equipment).

If more than one test method is available and applicable in phase 2, step 2 to substantiate a label claim for efficacy, it is sufficient to provide data from only one of the test methods. The test method selected should be one which best represents the way in which the product is used. For example, in the case of a disinfectant used for "hard, non-porous surfaces by spraying", the test method should be one for such surfaces without mechanical action and with representative conditions of use, such as contact time, soiling, temperature and test organisms.

It is not mandatory to perform the tests under obligatory test conditions of the standards if the claimed use conditions of the products are different from these obligatory test conditions.

Tests have to be performed with relevant target organisms, which are selected in accordance with the standard and the intended use of the product. This is further discussed in Section 1.3.1 of this Guidance. A list of standard test organisms is given in Appendix 3.

The concentrations used in testing should be selected to demonstrate the threshold of product efficacy. Suspension tests should be performed with several dose rates, including at least one rate lower than the effective rate. Competent Authorities (CAs) will evaluate dose-response data generated in these tests in order to assess if the recommended dose is appropriate (i.e. the concentration is not too high, or at the minimum) to achieve the desired effect.

For biocidal products which claim a biostatic effect (bacteriostatic, fungistatic, etc. i.e. the ability to inhibit the growth of bacteria, fungi etc. without killing them) the efficacy should be shown by suspension tests and simulated-use tests (e.g. surface tests). The suspension test and simulated-use test should be performed with and without neutralisation and with a water control (where water is tested instead of the product). The results from this testing should show that the product prevents the growth of the test micro-organism (i.e. a lower level of test organism compared to the water control) but does not necessarily inactivate them (the micro-organisms survive in the test without neutralisation).

Biocidal products that claim a biostatic effect bear the risk of the development of organisms with temporary or permanent reduced susceptibility (resistance). For this reason, the efficacy of these types of products has to be examined carefully.

In case of *in situ* production of the active substance or when an apparatus is used to dose the active substance in the right amount to the water, the report should contain information on safety measurements concerning over and under dosing.

Other products, which do not have biocidal or biostatic activity, might fall within the scope of the BPR, Article 3 1 (a) "*with the intention of destroying, deterring, rendering harmless, preventing the action of, or otherwise exerting a controlling effect on, any harmful organism by any means other than mere physical or mechanical action"* . No EU standards are available for these types of products yet, so applicants should provide a method following the principles of this guidance and based on scientific evidence. During the development of new tests, or when an applicant is considering using a non-standard test or using novel testing methods, they should discuss this with the CA as to the acceptability and applicability of the test.

In the following sections, guidance on the requirements per product type and use will be given.

Detailed but non-exhaustive lists of the most relevant product applications and uses of biocides, together with the required test methodology, are given in the claims matrices

which are a set of tables linked to this guidance document (see Appendix 1 for more information).

For uses and claims that are not specifically mentioned in this document, the requirements will be set on a case by case basis by the CA.

5.4.0.4.4 Relevant factors of the test procedure

Formulation of the tested product
A product authorisation is given to a single biocidal product with a defined composition or to a group of products making up a biocidal product family (BPF) and having similar uses, the same active substances, similar composition with specified variations and similar levels of risk and efficacy.

With respect to a single product, the efficacy of its specific formulation should be demonstrated. Therefore it is important that the formulation tested is clearly reported in each test report (or provided alongside the test report with a statement that it is the formulation which has been tested). The formulation details should specify the active substances and co-formulants present, together with their respective concentrations, and should confirm that all tested formulations contain the same co-formulants and concentrations. Any deviations should be mentioned and justified in a statement or in the relevant efficacy reports. Where there are deviations in the formulation from that in the product for which authorisation is sought, the tests will only be considered relevant where it is evident that the deviations have no positive effect on efficacy. In cases where this is not evident, a confirmatory study with the organisms that are most difficult to control should be proposed.

Within the BPF, the minimum level of efficacy over the whole potential range of products should be demonstrated and the permitted variations in composition and intended uses should be explicitly identified.

The test formulations should be chosen in such a way that they cover the whole potential range of products. The test formulations should include at least a product with the lowest concentration of the active substance. See also chapter 5.2 for more information on testing BPF.

Hard Water Claims
The degree of hardness of the water used to dilute the disinfectant may affect its performance (by the presence of metal ions such as Ca^{2+} and Mg^{2+}). Generally the harder the water is, the less effective the diluted disinfectant will be. Therefore, test programmes which require that products are diluted with potable water must be diluted in the water of standard hardness as defined in the corresponding test standard, for the purpose of efficacy testing.

It follows that any product that carries label claims for effectiveness in hard water must be tested by the appropriate method in water with defined hardness at the level claimed.

Presence of Interfering Substances
Where disinfectants are applied to either inanimate surfaces or skin or liquids, substances may be present on the surface or in the liquid, which may affect the disinfectant's activity.

The nature, amount and condition of the soiling present will affect the efficacy of a disinfectant.

In many cases, residual contamination must be expected and in some situations (e.g. in the treatment of blood spillages) disinfectants are specifically used to decontaminate soiling, to prevent infection transfer and to assist in safe disposal.

Blood, urine, faeces, food debris, fats and oils, dust and proteinaceous materials are the most likely organic soilings to be encountered. Limescale, milkstone and soil are the most common inorganic soilings.

Where claims are made for use under soiled or dirty conditions, the use concentrations of the product must be determined from tests performed in the presence of suitable soiling materials. Soiling materials commonly used in efficacy test methods include albumin serum, blood, yeasts and yeast extract.

In practice, with exception of a few situations (e.g. cleanrooms), the presence of soiling on surfaces or in liquids to be disinfected cannot be ruled out. For this reason, a small amount of interfering substance should always be included during the testing of the product. In the CEN methods, this is called "under clean conditions". Tests under clean conditions can be used when the surface is clean before disinfection. This is for instance the case when the label states that cleaning prior to disinfection is necessary. When a product claims combined cleaning and disinfection, the product should be tested under dirty conditions (see Appendix 4 for more information). Also, where the label only states excessive dirt should be removed, and the surface is still soiled after that (e.g. in the meat industry), soiling for dirty conditions should be used. Please note that in some cases EN 14885 is not always sufficient to meet BPR requirements.

When a product is to be recommended for certain uses where the soiling is of a specific type (such as soap film residue or hard water scum), the product must be tested in the presence of that specific soiling type. If more soiling types are relevant for the use of the product (e.g. a product must be used in the beverage industry, in the meat industry or in kitchens), pre-testing should be done to determine the most challenging soiling type. Extended testing with the most challenging soiling type will be sufficient to cover all the others.

As an exception to the rule, products to be used in cleanrooms do not require additional soiling in the test. A cleanroom has a controlled level of contamination that is specified by the number of particles per cubic meter at specified particle size. The soiling level in cleanrooms is so low that even testing under clean conditions for the EN tests is still over-dosing of soiling compared to cleanrooms. For these uses, the high load of test organisms can be seen as soiling. Tests without soiling will only be accepted when the label states the specific use in cleanrooms which are classified according to ISO 14644-1 in class 1 to 9 or according to GMP EU classification in Grade A to D.

Generally, soiling will reduce the efficacy of the disinfectant, and where soiling is present, longer contact times, higher concentrations, pre-cleaning or a combination of these elements may be necessary.

Temperature
Generally, disinfection performance increases with temperature, although this depends on the active substances and the effect on individual species may vary depending on the specific properties. Therefore, the test temperature should be representative of those encountered during the intended use of the product (e.g. low temperature in animal housing, higher temperature in CIP). Some biocides are used in chemothermal disinfection, for instance, some CIP treatments are done under temperatures of 60-80ºC. Also for these uses, the products should be tested at the use temperature.

If products (PT 2-4) are tested with high temperatures above 40ºC, heat resistant reference test organisms must be used[11]. *Enterococcus faecium* must be used as the only test organism for claiming bactericidal activity. For a virucidal claim, the only test organism must be *Murine Parvovirus*. For a sporicidal claim, the test organism can be spores of, for example, *Bacillus cereus* or *Clostridium sporogenes*.

For mycobacteria, yeasts and fungal spores no high temperature resistant standard test organisms are available. Most yeasts and fungal spores are already irreversibly inactivated by high temperatures (>40 ºC) in the control without an active substance. However, ascospores of several fungi can become heat resistant and can cause problems in, for instance, the food industry.

When standard tests with relevant temperature resistant strains become available for mycobacteria, yeasts and fungal spores, these should be used.

For specific claims against heat resistant species (e.g. *Talaromyces flavus*) efficacy tests with these organisms should be provided. In these tests, a control without biocide should be included which shows the survival of the test organisms at the high test temperature.

It is possible that the concentration needed to pass the test is higher for the organisms tested at low temperature than for the temperature resistant organisms tested at a higher temperature. In that case, a justification should be given on how the test results reflect the use concentration in the use instruction on the label.

Contact Time

The contact time of a product on a surface etc. is an important aspect in the evaluation of the efficacy of disinfectants. In general, the longer the contact time, the more effective the disinfectant is. In trials where test organisms are taken from treated samples for further analysis, the contact time between the biocide and the test organisms should be stopped. Neutralisers, membrane filtration or subculture techniques are used to prevent residual carry-over of active substances. Neutralisation is discussed further in section 1.4.4.6 of this Guidance.

Some disinfectants act very quickly, whereas others require an extended contact time to achieve adequate performance. Mycobacteria, bacterial spores, fungal spores and non-enveloped viruses take longer to be irreversibly inactivated than most vegetative micro-organisms.

The contact time that is practical in real life use should be taken into consideration when testing. In phase 2 and phase 3 tests the product should pass the test at the contact time recommended on the product label.

Neutralisation

Neutralisers are used to stop the product's activity in trials where the test organisms are taken from treated samples for further analysis, such as plate count following biocidal treatment. An effective neutraliser for the test product should be identified, and evidence demonstrating the effectiveness of the neutraliser against the product under test, and showing that the neutraliser itself does not have antimicrobial activity, must be included in a test report. Membrane filtration or subculture techniques can be used to stop the product's activity, in combination with or instead of chemical neutralisation. These other methods are covered by the term "neutralisation" as used in this guidance.

[11] See also Technical Agreements for Biocides – Efficacy (EFF-TAB): Efficacy testing for disinfectants at elevated use temperatures

Appropriate controls for determining the efficacy of the procedure to stop the product's activity after the contact time should be performed.

pH
The prevailing degree of acidity or alkalinity during disinfection can also affect the performance and choice of disinfectant. Therefore, the pH of the product at the use concentration (diluted) as used in the test must be included in the test report.

Texture of Surfaces
Smooth impervious surfaces are easier to disinfect (and also to clean) than rough or pitted ones. In some circumstances, the micro-organisms might be protected from the action of disinfectants by being protected in porous surfaces. Clumps of micro-organisms may also be more difficult to inactivate, as cells inside are protected by dead micro-organisms on the outside. Recently porous surface tests have been developed (CEN) to test under these conditions.

Bacteria and fungi can adhere to surfaces forming biofilms. In biofilms, susceptibility is decreased (the bacteria are in a different physical state) and penetration of biocide can be difficult to achieve due to the matrix surrounding the bacteria. This makes bacteria in a biofilm more difficult to inactivate.

Repetition
In general test results become more reliable when the tests are done in replicates (e.g. repeated in time, in more test objects). Replicates should be performed as required in the appropriate EN standards and where appropriate, internal standards or reference substances should be included.

EN 14885 section 5 (parts b, c and d) state the following information on the precision of the test methods (repetitions):

- For standardised tests, or adaptation of a standard test, it is recommended to repeat the test and/or include an internal standard and/or performing the test in a second and/or third laboratory. When doing the latter the second laboratory (and any further laboratory) might only repeat the test which is regarded as the most relevant one with the least susceptible test organism(s). If results from two or more laboratories are used, each laboratory has to specify one result, e.g. "R = > 5.2 lg (EN 13727-instrument disinfection)". Then the mean of the results of all laboratories is calculated assuming each laboratory's result as equivalent. Results with lg "more than" are set as this figure, e.g. "> 5.2 lg" is used for calculation as "5.2 lg". All lg values are converted to real numbers, e.g. 5.2 lg to about 158.000. The mean is the arithmetic mean of these converted numbers. If one of the testing laboratories obtains a result less than the required lg reduction, the product must pass if further tests by three other laboratories demonstrate a pass. The calculations above cannot be done with tests where pass criteria are not expressed as lg reduction.
- In case of repetition of the test it is unnecessary to repeat the test with all test-organisms but only with the least susceptible to the product under test.
- If two or more tests are carried out to support a claim of performance (e.g. phase 2, step 1 and phase 2, step 2) and the ensuing recommendation for use, the tests may be ranked according to their order of relevance, i.e. their ability to predict the product's performance under real life conditions. In case of a ranking only the result of the most relevant test may be repeated taking into account advice c). If a ranking is not possible only the results of the test showing the highest minimum active concentration should be repeated.

5.4.0.4.5 Co-formulant(s) being a potential active substance[12]

This section gives guidance on how to identify additional active substances. For guidance on how to identify the influence of co-formulants, e.g. in the course of the identification of a worst case test product for a BPF please refer to the BPC-37 document *"Harmonized approach to determine a worst-case (or a representative) test product to be taken into account for efficacy core assessment for a disinfectant BPF"*[13].

In case during the evaluation phase of a biocidal product containing one or more co-formulant(s) where the CA regards one or more of the co-formulant(s) to be an additional active substance, the applicant should provide an explanation on its function in the formulation together with the justification why this substance is not considered as an active substance. Only in cases where a justification is not conclusive should tests be provided to demonstrate the 'non-activity' of the co-formulant(s).

The following strategy has been developed:

Three kinds of tests have been identified. The CA may request one, two or all of them – as necessary and appropriate.

Test 1: The biocidal product without an active substance is tested.

The active substance(s) are replaced by water or, when justified, any other suitable substance(s). The test should be performed at the recommended concentration of the product.

If the active substance(s) cannot be replaced for whatever reason, the concentration of the product without the active substance has to be decreased accordingly[14].

In cases where in this test a high lg reduction is seen, further test 2 with each co-formulant under question would be required to verify which co-formulant is causing this effect.

Test 2: Each co-formulant under question is tested alone.

The concentration (of the co-formulant) in the test has to be adapted to the relative amount of the co-formulant in the biocidal product[15].

Test 3: The biocidal product without the co-formulant is tested.

Two products are tested in parallel: the biocidal product and the same product, but without the co-formulant that should be replaced by water or, when justified, any other suitable substance(s). Separate testing may be performed for each co-formulant under question removing only one co-formulant at a time. The test should be performed at the recommended concentration of the product.

Any deviation from the test method above must be clearly described and justified.

Generally, these tests should be performed with bacteria.

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[12] Please see also CA-Jan18-Doc.4.2_final: Assessment of the efficacy role of some co-formulants_final

[13] See BPC-37

[14] Example: Amount of the active substances is 30g/100g in the biocidal product. Concentration used for claiming bactericidal activity is 2.0 %. Concentration in Test 1 should be 2% of 70.0g = 1.4 %.

[15] Example: Amount of the co-formulant is 3.0g/100g in the biocidal product, concentration used for claiming bactericidal activity is 3.0 %, concentration of the co-formulant in Test 2 should be 3% of 3.0g=0.09 %.

Each test should be performed as a (modified) phase 2, step 1 test. For all tests it is requested to show a definite lg reduction considering the detection limits of the respective tests, i.e. within the detection limits precise lg reduction values need to be given such as 2.68 lg instead of <5.00 lg. The EN tests may be adapted accordingly, if necessary. For instance, extra dilution steps will be needed for these tests to show lg reductions around 3.00 and 3.50.

To demonstrate in tests 1 and 2 that the co-formulants under question are not active substances, the lg reduction should be at least 2 lg lower than the required lg reduction in the EN phase 2 step 1 test performed. For test 3, the lg reduction of the two products should be similar, i.e. show no more than 1.50 lg difference.

Since both tests 1 and 2 are tests without active substance, the conditions should not be as severe as under use conditions. These phase 2 step 1 tests should be performed with a proportionate amount of interfering substance and with the longest contact time claimed for the product.

Test 3 should be performed under the test conditions (interfering substance/soiling, contact time) used for a product claim, demonstrating that the product without the co-formulant is still efficacious under use conditions.

In all tests the pH of the test solution should be adjusted to the pH of the biocidal product.

Schematic overview of possible test results and conclusions:

Table 7. Three kinds of test types for identifying additional active substances.

Test	Test product*	Result (lg reduction)	Conclusion
Test 1	BP without AS	<3**	all CFs are not active substances in this product
		≥3**	one or more or the combination of the CF might have biocidal activity in the product
Test 2	Only CF	<3**	this CF is not an active substance in this product
		≥3**	this CF might be acting as an active substance in this product
Test 3	BP without CF	≥3.5**	this CF is not an active substance in this product
		<3.5**	this CF might be acting as active substance

* BP = biocidal product; AS = active substances; CF = co-formulant.
** lg reduction in an EN phase 2 step 1 tests for bacteria (EN 1276; EN 13727; EN 1656).

5.4.0.4.6 Efficacy testing of stored disinfection products to determine/confirm the shelf life

If the active substance concentration decreases by more than 10% during the shelf life of the biocidal product, efficacy tests should be performed to confirm the shelf life by

demonstrating the efficacy of stored product[16]. In general, efficacy shelf life tests are acceptable if at least one of the following issues is addressed:

- Efficacy shelf life tests should preferably be performed with aged products that have been stored for the complete claimed shelf life, or alternatively, with the accelerated aged products with active substance concentration comparable to concentration measured in the stored product after the claimed shelf life.
- In some cases, it is also acceptable when efficacy shelf life tests are performed with a fresh product with an active substance concentration comparable to the concentration measured in a stored product after the claimed shelf life. In those cases, a robust justification and/or a clear indication from the physico-chemical assessment is required which explains why age-related changes in co-formulants would not have an effect on the efficacy of the aged product, and why the reduction in the quantity of active substance would be the only issue to be addressed.

An efficacy shelf life test can be a phase 2, step 1 test. The test can be performed with the most tolerant test organism within the claimed target organism group which is most difficult to kill, under the most difficult conditions (a robust justification should be provided based on the fresh product data). The most difficult conditions are the ones for which the highest product dose is required.

5.4.0.5 General data requirements

5.4.0.5.1 Test range

Tests (phase 2, step 1) should be performed at a range of concentrations in order to verify that the use concentration is suitable for the desired effect, e.g. not too high or not at the minimum effective level.

5.4.0.5.2 Claim for several areas of use

In cases where the product is intended for several areas, it is usually acceptable to perform the tests from only one area, as long as the test is performed with the worst case test conditions (temperature, lg reduction, interfering substances, etc.) and the test with the highest/most stringent pass criteria is used. In case the strains are different between the PTs all the strains must be tested.

5.4.0.5.3 Biocidal products with biostatic effect

For biocidal products with a biostatic effect (bacteriostatic, fungistatic, etc.), the efficacy should be shown by suspension tests and simulated-use tests (e.g. surface tests). The suspension test and simulated-use tests should be performed with and without neutralisation. The results from these tests should show that the product prevents growth of the test organism (no increase in numbers compared to the negative control) but does not necessarily inactivate them (survival of the test organism in the test without neutralisation).

5.4.0.5.4 Malodour control

There are specific requirements for products claiming control of organisms that cause malodour. Phase 2, step1 and step 2 tests should be performed with odour producing micro-organisms. A justification for which bacteria, fungi, etc. are relevant to the intended use should be provided. Along with these laboratory tests, an odour test should be performed. The CA will decide on a case-by-case basis whether the product will receive authorisation.

[16] See Volume I Parts A+B+C, and Technical Agreements for Biocides APCP, point 4.2.1

5.4.0.5.5 Changes in ingredients [17]

When small changes are made to the non-active ingredients in a product, it is not always necessary to repeat all the tests with the new formulation. The applicant may provide a description of the changes and the effects that they have on the efficacy of the product. In the case of a minor change, a robust justification might be sufficient (to be decided by the CA). In other cases, new efficacy tests will have to be provided. This can be either a full set of efficacy tests or a test with the least susceptible organism in the former test.

5.4.0.5.6 Treated articles

See Section 5.3 for guidance on Treated Articles.

5.4.0.5.7 Biocidal Product Families

When authorisation is requested for a product family, efficacy should be demonstrated for the whole group but not necessarily of each product. More information is available in Section 5.2 Product Families.

5.4.0.6 Resistance

See section 3.2 for guidance on resistance.

5.4.0.7 Assessment of application for authorisation

5.4.0.7.1 Decision making

Biocidal Product Regulation 528/2012 (Annex VI) stipulates rules for decision making for biocides.

The test results must meet the requirements of the standards or other criteria for acceptance which are described below per type of use. Where a product does not conform to these criteria, the applicant should provide a justification in the application as to why the product should still be recommended for authorisation. The CA will decide on a case-by-case basis whether the product will receive authorisation.

5.4.0.7.2 Assessment

The CA assessor/expert assesses the performance of the product as demonstrated in the submitted efficacy tests against the label claims made for the product and the above criteria. If the product is judged to be sufficiently effective in laboratory (and, where relevant, field) tests, the product will be recommended for authorisation as far as efficacy is concerned.

In exceptional cases the applicant may provide justification as to why the specified acceptance criteria are not met but the product is still acceptable. The CA will evaluate any justification on a case-by-case basis, possibly in consultation with the other CAs, and decide whether it is acceptable or not.

The following sections give more specific dossier requirements per type of disinfectant.

[17] For this section, the product family concept of the BPR is not yet taken into account.

5.4.1 PT1 Human hygiene biocidal products

5.4.1.1 Introduction

Product type 1 contains biocidal products used for human hygiene purposes, applied on or in contact with human skin or scalps for the primary purpose of disinfecting the skin or scalp.

Products applied on human skin may be assigned to either biocidal, medicinal or cosmetic products or even to medical devices. If the product under investigation is within the scope of the Medicinal Products Directive (2001/83/EC), the Cosmetic Products Regulation ((EC) No 1223/2009) or the Medical Devices Directive (93/42/EEC), it is excluded from the BPR for the respective use.

Products for disinfection of damaged skin (e.g. wound disinfection) or disinfection of undamaged skin before a medical treatment of a patient (e.g. pre-operative skin disinfection before surgery and disinfection before injection) and products with a claim of medicinal use are always medicinal products (covered by the Directive 2001/83/EC on medicinal products for human use).

Biocidal products within PT1 are mainly hand disinfectants, which can include disinfection of the wrist and forearm.

When applying for authorisation for a biocidal product within PT1 a detailed description of the intended use should be given, to prevent authorisation of medicinal products, or cosmetic or medicinal uses, as biocides (e.g. the claim "skin disinfection" is insufficient).

For products that fall under the BPR the data requirements described in the following sections apply.

5.4.1.2 Hand disinfectants

5.4.1.2.1 Introduction

Hand disinfectants can be divided into hygienic handwash, hygienic handrub, surgical handwash and surgical handrub products. Handwash products are intended to be used with water, handrub products are intended to be used without the addition of water. Hand disinfectants can include disinfection of the wrist and forearm. Products include liquids, gels, wipes, etc.

Hand disinfectants can be used in a wide variety of areas such as hospitals and other healthcare institutions, food, beverage and other industry, private homes, etc.

In the sections below the requirements and acceptance criteria for most common uses are specified. For other uses and claims that are not specifically mentioned the requirements will be set on a case-by-case basis by the CAs.

5.4.1.2.2 Data requirements

Test methods

For efficacy testing of hand disinfectants, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred. For hygienic handwash, hygienic handrub, surgical handwash and surgical handrub phase 2, step 1 and step 2 tests are required. Phase 2, step 1 tests are available for all relevant test organisms and required depending on the claim made. For a claim without specification of the area of use the phase 2, step 1 for the medical area should be used.

For bacteria a phase 2, step 2 test is available for these uses and therefore mandatory. For other organisms phase 2, step 2 tests will be mandatory when they become available. For an overview of available EN tests see Appendices 2 and 4.

Disinfectant towelettes/wipes
For hand disinfectant wipes, phase 2, step 1 tests should be done preferably with the liquid extracted from the wipe or, if difficult to extract, use the liquid as it is before it is added to the wipes. Phase 2, step 2 tests for hand disinfection (modified EN 1500) should be tests with the wipe applied on volunteers' hands according to the intended use. The wipes should be used on full hands and not on the fingertips only. In addition, a test must be performed that shows that either the wipe will still disinfect if the wipe dries out, or that the wipe stays wet long enough to disinfect according to the claim. In addition, the use directions can address these issues, for instance, stating on the label that only wet wipes are efficacious or giving expiry dates for re-sealable packages if appropriate according to the intended use conditions.

Test organisms

Hand disinfectants intended for general hygiene purposes should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided. For hand disinfectants intended for use in industrial environments to prevent spoilage of products, in some cases also prevention of bacteria and yeast infections is of importance, for example, in the food and cosmetic industry. In other industries, it may be justified that only efficacy against bacteria is sufficient.

For all other groups of organisms, tests have to be provided only when activity against those specific organisms is claimed.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods.

Additionally to the obligatory species, other species can be used if there are valid scientific arguments to justify their use, such as a need to show activity against specific organisms of concern in a human health environment, especially emerging health risks, or in specified industries.

An overview of reference test organisms is given in Appendix 3.

Virucidal activity
For products used as hygienic hand disinfectants, a differentiation in the virucidal activity is made.

The claims can be:

- virucidal activity;
- limited spectrum virucidal activity;
- virucidal activity against enveloped viruses.

For each claim, different test organisms should be tested, i.e. for virucidal activity Poliovirus, Adenovirus and Murine Norovirus, for limited spectrum virucidal activity Adenovirus and Murine Norovirus, and Vaccinia virus for activity against enveloped viruses.

The SPC should clearly state which virucidal claim is demonstrated. When only the limited spectrum virucidal activity or virucidal activity against enveloped viruses is demonstrated, the claim cannot be "virucidal activity".

General public (non-professional users) may not understand the difference between a virucidal activity claim, a limited spectrum virucidal activity claim and a claim against enveloped viruses. Therefore, the instructions for general public should be carefully worded.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC.

The contact time can be found in the relevant EN standards. In general:

- for hygienic handwash and handrub products the contact time is between 30 and 120 seconds;
- for hygienic handwash and handrub products used in the medical area the contact time is usually 30 seconds for bactericidal, yeasticidal activity and virucidal activity against enveloped viruses;
- for surgical hand disinfection products the contact time should not exceed 5 minutes.

It must be assured that the disinfected hands stay wet during treatment, e.g. by applying enough product or by applying the product several times.

Phase 2, step 1 tests should be carried out with soiling (interfering substances) for clean or dirty conditions depending on the intended use and according to the relevant EN tests, i.e. EN 13727 and EN 13624 (medical and veterinary area) or EN 1276 and EN 1650 (industrial, domestic, institutional area). Dirty conditions in phase 2, step 1 tests are mandatory for handwash applications. For handrubs, clean conditions in phase 2, step 1 tests suffice if use instructions state that the product must be applied on visibly clean hands.

For handwash products, the phase 2, step 1 tests should be performed with pre-diluted product to take into account that the product is used on wetted hands. This is described in EN 13727, EN 13624, EN 1276, and EN 1650, and a similar approach should be taken for other organisms.

Phase 2, step 2 tests are performed without additional soiling according to EN 1499, EN 1500 or EN 12791. The soiling needed for clean and dirty conditions can be found in the relevant EN phase 2, step 1 tests and in the Appendix 4 . Note that dirty conditions for products used in hospitals and healthcare differ from those in other areas of use. It is important that the tests are performed using the same contact time as claimed in the SPC.

The contact time can be found in the relevant EN standards. In general:

- for hygienic handwash and handrub products the contact time is between 30 and 120 seconds;
- for hygienic handwash and handrub products used in the medical area the contact time is usually 30 seconds for bactericidal, yeasticidal activity and virucidal activity against enveloped viruses;
- for surgical hand disinfection products the contact time should not exceed 5 minutes.

It must be assured that the disinfected hands stay wet during treatment, e.g. by applying enough product or by applying the product several times.

Phase 2, step 1 tests should be carried out with soiling (interfering substances) for clean or dirty conditions depending on the intended use and according to the relevant EN tests, i.e. EN 13727 and EN 13624 (medical and veterinary area) or EN 1276 and EN 1650 (industrial, domestic, institutional area). Dirty conditions in phase 2, step 1 tests are mandatory for handwash applications. For handrubs, clean conditions in phase 2, step 1 tests suffice if use instructions state that the product must be applied on visibly clean hands.

For handwash products, the phase 2, step 1 tests should be performed with pre-diluted product to take into account that the product is used on wetted hands. This is described in EN 13727, EN 13624, EN 1276, and EN 1650, and a similar approach should be taken for other organisms.

Phase 2, step 2 tests are performed without additional soiling according to EN 1499, EN 1500 or EN 12791. The soiling needed for clean and dirty conditions can be found in the relevant EN phase 2, step 1 tests and in the Appendix 4. Note that dirty conditions for products used in hospitals and healthcare differ from those in other areas of use.

5.4.1.2.3 Acceptance criteria

A product in PT1 will be assessed to be sufficiently effective if the required laboratory tests have been carried out (using the required test organisms and test conditions), and the pass criteria for the tests have been met.

Where pass criteria are available in the standard test, these should be met. For PT1 products the required lg reductions are referenced in Appendix 4 or EN 14885.

Since the test methods for these types of products are generally established, deviations are not foreseen. If, however, deviations are considered necessary, they must be justified in the application.

5.4.1.3 Other skin and scalp disinfection

For other skin and scalp disinfection products the overlap with medicinal and cosmetic products is significant. Only products that are not covered under either of these directives can be considered as PT1 disinfectants.

5.4.1.3.1 Data requirements

Test methods

For other skin disinfection products, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred: phase 2, step 1 and step 2 tests are required.

The same phase 2, step 1 tests as required for hand disinfectants can be used.

Currently, there are no European phase 2, step 2 standard tests available for other skin disinfectants. However, test protocols may be designed by adapting existing standards (e.g. CEN methods involving volunteers) in a way that mirrors the respective application.

Newly designed test protocols should be timely discussed with and agreed upon by the CA before tests are carried out.

Deviations from the existing/future standards should always be mentioned and justified.

For an overview of available tests see Appendices 2 and 4.

Test organisms

Disinfectants for other skin and scalp should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For phase 2, step 1 tests the standard organisms of these tests should be tested.

For phase 2, step 2 tests either the standard organisms of these tests can be tested or the normal occurring microflora in volunteer tests.

For all other groups of organisms tests only have to be provided when activity against those specific organisms is claimed.

Justification for the used test organisms should be provided.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference test organisms is given in Appendix 3.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value and should be justified for the use.

Phase 2, step 1 and phase 2, step 2 tests should be carried out with BSA as soiling (interfering substances) for clean or dirty conditions depending on the intended use. Simulated-use studies with volunteers are in general performed without additional soiling.

The soiling needed for clean and dirty conditions can be found in the relevant EN tests (see EN 14885, medical area) and referenced in Appendix 4.

5.4.1.3.2 Acceptance criteria

A product in PT1 will be assessed to be sufficiently effective if the required laboratory and simulated-use tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test, these should be met. For PT1 products the required lg reductions are referenced in Appendix 4 or EN 14885.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.2 PT2 Disinfectants and algaecides not intended for direct application to humans or animals

5.4.2.1 Introduction

Product type 2[18] contains disinfectants and algaecides not intended for direct application to humans or animals. This includes *inter alia:*

- products used for the disinfection of surfaces, materials, equipment and furniture which are not used for direct contact with food or feeding stuffs;
- usage areas such as swimming pools, aquariums, bathing and other waters; air-conditioning systems; and walls and floors in private, public, and industrial areas and in other areas for professional activities;
- products used for disinfection of air[19], water not used for human or animal consumption, chemical toilets, waste water, hospital waste and soil;
- products used as algaecides for treatment of swimming pools, aquariums and other waters and for remedial treatment of construction materials;
- products used to be incorporated in textiles, tissues, masks, paints and other articles or materials with the purpose of producing treated articles with disinfecting properties.

[18] This includes biostatic products.

[19] This is taken to mean disinfection of air itself. Disinfectants sprayed or vaporised into the air (e.g. room disinfection by vaporised biocide) are generally for the purpose of disinfecting surfaces and not the air itself. Disinfectants for air conditioning systems disinfect the surfaces in these systems, not the air coming out of it.

The data requirements (test standards and test organisms) and assessment criteria for the most common uses are specified below. Detailed but non-exhaustive lists of the most relevant product applications and uses of disinfectants within PT2, together with the relevant test methodologies are given in the claims matrices which are a set of tables linked to this guidance document (see Appendix 1 for more information).

All of the possible uses in this PT cannot be covered in the matrices. For other, less common, uses and claims that are not specifically mentioned, there is often no international standard tests available. Where this is the case, the applicant should provide tests that show the efficacy of the product and a justification for the use of these tests. The assessment of these products will be based on expert judgement and will be handled case-by-case.

5.4.2.2 Data requirements

There are some general data requirements that apply to all uses in PT2, and these are described below. There are also specific data requirements that apply to different types of use, and these are described in the sections covering those uses.

The intended uses of the disinfectant determine which tests will be required to support the product. Tests that most closely reproduce the practical application conditions should be selected.

In general it is not known which organisms are present on a surface or matrix to be disinfected. Therefore a disinfectant must have a broad spectrum of activity, in order to control all of the organisms that may be present.

5.4.2.2.1 Use in health care

For general applications in healthcare areas, e.g. disinfection of surfaces, the products should be at least sufficiently effective against bacteria and yeasts (which are responsible for the most common nosocomial infections). Additionally, efficacy against other organisms can be claimed.

Products intended to disinfect surfaces that are frequently touched and cannot be kept free from touching, either by patients, medical staff or different people, longer than 5 min (therefore, potentially leading to the transmission of microorganisms to the patient), requires a maximum contact time of 5 min. In case when activity against more challenging target organisms (mycobacteria, viruses, fungal spores, bacterial spores) is claimed, which need a longer contact time, then a maximum 15 min is possible. Products for other surfaces that should be kept free from touching, e.g. by locking a room, may be tested with a contact time of maximum 60 min.

5.4.2.2.2 Tuberculosis departments

If the product is to be used in tuberculosis departments, the product should be efficacious as a general disinfectant used in health care (efficacy against bacteria and yeasts), but tuberculocidal activity or mycobactericidal activity must also be demonstrated.

5.4.2.2.3 Cleanrooms

Products to be used in cleanrooms only differ in the data requirements for the interfering substance to be used in the tests. As an exception to the rule, products to be used in cleanrooms do not require additional soiling in the test. A cleanroom has a controlled level of contamination that is specified by the number of particles per cubic metre at specified particle size. The soiling level in cleanrooms is so low that even testing under clean conditions for the EN tests is still overdosing of soiling compared to cleanrooms. For these uses, the high load of test organisms can be seen as soiling. Tests without soiling will only

be accepted when the label states the specific use in cleanrooms which are classified according to ISO 14644-1 in class 1 to 8 or according to GMP EU classification in Grade A to C.

5.4.2.2.4 Products against viruses

Products against viruses must be effective against viruses with and without an "envelope" (protein or lipid mantle). For such products, virucidal activity can be claimed if efficacy against non-enveloped viruses has been proven.

For products used as hard surface disinfectants, a differentiation in the virucidal activity can be made.

The claims can be:

- virucidal activity;
- limited spectrum virucidal activity;
- virucidal activity against enveloped viruses.

For disinfectants used by professional users, the virucidal activity, limited spectrum virucidal activity and the virucidal activity against enveloped viruses can be claimed.

For disinfectants used by the general public in non-healthcare areas, only the virucidal activity and virucidal activity against enveloped viruses can be claimed.

For each virucidal claim, different test organisms should be tested as specified in the relevant EN standards (see Appendix 3).

5.4.2.3 Disinfectants for hard surfaces (in PT2)

5.4.2.3.1 Introduction

Biocides can be used to disinfect hard surfaces in areas such as hospitals (including veterinary hospitals, dental facilities etc.), industry, institutions or private homes. These surfaces may be tables, floors, walls, the outsides of machinery and hard furniture, etc. Products are often wiped or sprayed onto the surfaces and may be washed or wiped off after a certain contact time.

The testing requirements for some specific uses of hard surface disinfectants are discussed in separate sections, for example, toilets, room disinfection with vaporised biocide, immersion of equipment into the product, etc. As the areas of use can be as diverse as private homes to operating theatres, the test requirements might vary depending on the area of use.

5.4.2.3.2 Data requirements

See general data requirements for PT2. A detailed, but non-exhaustive list of the most relevant product applications and uses of hard surface disinfectants and the required test methodologies are given in Claims Matrix PT2, table for "Hard surfaces": the claims matrices are a set of tables linked to this guidance document (see Appendix 1 for more information and also Appendix 4).

Test methods

For efficacy testing of hard surface disinfectants, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a hard surface disinfectant:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative surface test (phase 2, step 2),

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Table 8. The following phase 2, step 2 tests should be used for the respective application methods.

Application group	Application method	Exemplary use instructions	Phase 2, step 2 test	Wipe/mop test material
Spraying	1. Spraying 2. Pouring 3. Foaming	Make sure to wet surfaces completely by spraying the product onto the surface to be disinfected. After spraying, the required contact time has to be respected until further treatment, e.g. wiping to dry the surfaces.	Tests without mechanical action, e.g. EN 13697 or EN 17387	not applicable
Wiping with specified wipes	Wiping with ready-to-use wipes.	Wipe the surface to be disinfected. Make sure to wet surfaces completely.	Test with mechanical action, e.g. EN 16615	Specified wipe material. In case several wipe materials are included, testing should at least be carried out with a representative worst-case wipe material (the choice of worst-case material needs to be justified). If this is not feasible, at least one wipe material should be tested with all test organisms and the remaining wipe materials at least with the most resistant test strain of each target organism group.
	Wiping with specified wipes, which are soaked on-site by the user.	Soak the wipes with product/spray product on the wipe until completely soaked and then wipe the surface to be disinfected. Make sure that the surface is completely wet after the wiping step. Example: specified wipes are provided in dry form in a bucket. Prior to use, the user pours the liquid product in the bucket and lets the wipes soak	Test with mechanical action, e.g. EN 16615	
Wiping with unspecified wipes	Applying product onto wipe followed by wiping	Apply, e.g. spray, pour product onto wipe until it is soaked and then wipe the surface to be disinfected. Ensure that the surface is completely wet after the wiping step.	Test with mechanical action, e.g. EN 16615	Testing should be carried out with the standard wipe listed in EN 16615.

	Applying product onto surface followed by wiping	Apply, e.g. spray, pour product onto the surface to be disinfected and then wipe the surface. Ensure that the surface is completely wet after the wiping step.		
Mopping with specified mops	Mopping with ready-to-use mops	Mop the surface to be disinfected. Make sure to wet surfaces completely.	Test with mechanical action, e.g. EN 16615	Testing should be carried out with the standard wipe listed in EN 16615.
	Mopping with specified mops, which are soaked on-site by the user.	Soak the mop with the product and then mop the surface to be disinfected. Ensure that surface is completely wet after the mopping step.	Test with mechanical action, e.g. EN 16615	
Mopping with unspecified mops	Applying product onto mop followed by mopping	Soak the mop with the product and then mop the surface to be disinfected. Ensure that surface is completely wet after the mopping step.	Test with mechanical action, e.g. EN 16615	Testing should be carried out with the standard wipe listed in EN 16615.
	Applying product onto surface followed by mopping	Apply, e.g. spray, pour product onto the surface to be disinfected and then mop the surface. Ensure that surface is completely wet after the mopping step.		
Others	Brushing	Make sure to wet surfaces completely when applying the product onto the surface by brushing.	Tests without mechanical action, e.g. EN 13697 or EN 17387	not applicable

Tests in phase 3 are optional, as no validated test methods are available yet. Several methods for testing the efficacy of hard surface disinfectants are available. Appendices 2 and 4 give a list of recommended test methods.

The following documents are recommended for surface disinfection:

- EN 14885: gives an overview of which EN phase 2, step 1 and step 2 tests to use for different uses,

if CEN standards are not relevant or available for the use or organisms claimed, the following documents are recommended if appropriately reflecting the application:

- OECD guidance for the testing of chemicals: Quantitative methods for evaluating the activity of microbicides used on hard non-porous surfaces (these are surface tests which would be considered phase 2, step 2 tests),

The use of the specified tests is strongly recommended where they are relevant and appropriate. Where the tests are not appropriate to the product, other tests can be used, although a justification for the relevance of the tests used should also be provided.

It is preferred that tests should be selected that correspond to the use area of the product (e.g. tests from medical areas for use in hospitals and tests from industrial areas for use in the cosmetic industry). Where the product is intended for use in several areas it is acceptable to perform the tests specified for only one of the areas, as long as the test with the highest/most stringent pass criteria is used. In some cases where the worst-case cannot be clearly identified all areas must be tested.

Where specific conditions apply for a field of use, such as high/low-level soiling, high/low temperatures, relevant contact times, etc. (see section 5.4.0.4.4 of this Guidance), these conditions should be included in the efficacy testing.

Disinfectant towelettes/wipes

For disinfectant wipes, the phase 2, step 1 tests should be done preferably with the liquid extracted from the wipes, or if difficult to extract, using the liquid as it is before it is added to the wipes. Phase 2, step 2 tests should be tests with mechanical action. These tests are available for bacteria and yeasts. For testing other organisms surface tests can be done with liquid extracted from the wipes (not the original liquid), with a justification of the volume that is applied per square centimetre. In addition, a test must be performed that shows that either the wipe will still disinfect after the wipe dries out, or that the wipe stays wet long enough to disinfect according to the claim. In addition, the use directions can address these issues, for instance, stating on the label that only wet wipes are efficacious, defining the surface area each wipe can disinfect (e.g. 0.5 m^2), or giving expiry dates for re-sealable packages.

Test organisms

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. For use in veterinary healthcare the target organisms in the test for the veterinary area (PT3) should be used.

If standard tests are not used (there will normally need to be a justification for this), the test organisms used to support a general claim should be demonstrated to be equivalent to the reference test organisms given in Appendix 3.

Tests with test organisms other than those mentioned in Appendix 3 are acceptable if adequate scientific evidence is submitted on which the relevance of the test organism to the field of use can be judged.

Also, see the general data requirements PT2 for specific claims and minimum requirements in healthcare.

5.4.2.3.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met.

If the test does not provide these criteria, the general criteria referenced in Appendix 4 or EN 14885 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.2.4 Soft furnishings

5.4.2.4.1 Introduction

Disinfectants for use on soft furnishings are intended to be used on fabrics in the home, institutional environment, healthcare and healthcare facilities. These can be used to treat porous soft surfaces such as curtains, sofas, upholstery, mattresses and carpets. The products are often sprayed onto the surfaces.

5.4.2.4.2 Data requirements

See general data requirements for PT2. A detailed, but non-exhaustive list of the most relevant product applications and uses of soft furnishing disinfectants and the required test methodology is given in Claims Matrix PT2, table for "Soft furnishings": the claims matrices are a set of tables linked to this guidance document (see Appendix 1 for more information).

Test methods

For efficacy testing of surface disinfectants for use on soft furnishing the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a surface disinfectant:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative surface test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Tests in phase 3 are optional as no validated test methods are available yet.

Where possible, the phase 2, step 1 test should be selected from EN 14885 from the table that corresponds to the use area of the product (e.g. test from the medical area for use in hospitals and test for domestic areas for use in private homes).

The phase 2, step 2 surface carrier test can be derived from the adaptation of CEN TC 216 surface tests. Instead of a hard surface carrier, carriers could be made of suitable fabric types. ISO 20743 can also be used, or other quantitative methods including textile as a carrier. EN 16616 is not relevant since this is done in washing machines.

Test organisms

The same test organisms as given for hard surfaces should be tested. See section 5.4.2.3.2 test organisms, of this Guidance and Appendix 3.

5.4.2.4.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests, these should be met.

If the test does not provide these criteria, the general criteria referenced in Appendix 4 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.2.5 Room disinfection/automated airborne disinfection of surfaces

5.4.2.5.1 Introduction

Automated airborne room (enclosure) disinfection involves the reduction and inactivation of micro-organisms on the surfaces of the walls, floors and ceilings of the room, as well as on external surfaces of the furniture and equipment present in the treated room. The product is applied by airborne diffusion in the form of an aerosol, a vapour or a gas, generated from a device, without the need for human intervention. A homogeneous distribution of the biocidal product in the volume of the room, reaching all surfaces (including ceilings and the undersides of horizontal surfaces), needs to be ensured. Manual or other ways of directed spraying are not covered in this section, but under hard surface disinfection (see section 5.4.2.3 of this Guidance).

Room disinfection may not disinfect the inside parts of furniture, and will not disinfect the air itself, so these uses are not considered in this section.

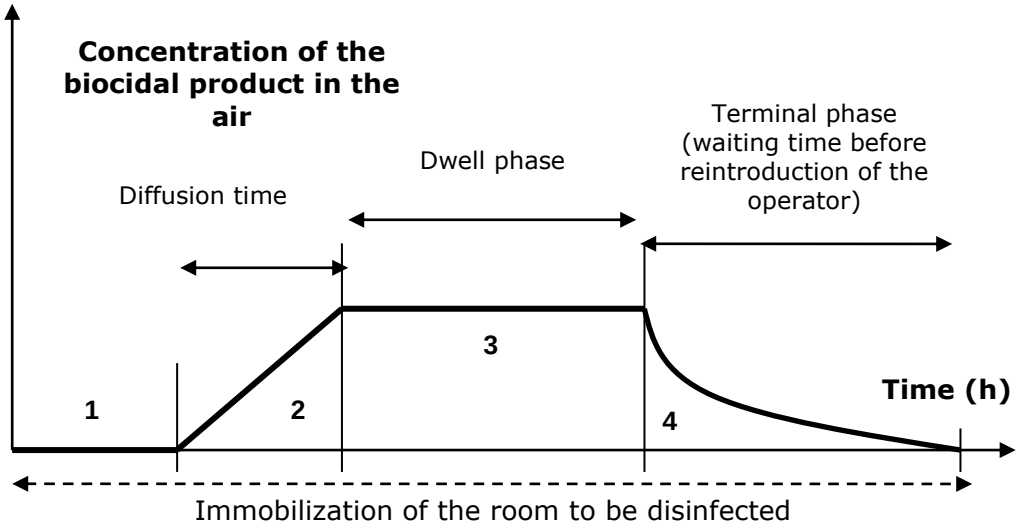
5.4.2.5.2 Process

The application of the product consists of four phases:

1. the conditioning phase (required depending on the type of active substance and application procedure), during which the environmental conditions (relative humidity, temperature) are modified to an optimal level for the product;
2. the gassing/diffusion phase, during which the product is diffused into the room (enclosure), in order to reach the effective concentration (diffusion time);
3. the dwell phase, which corresponds to the contact time required to obtain the expected level of efficacy;
4. the terminal phase, which includes aeration of the room to remove any disinfectant present in the air, or other procedures for inactivation of the active substance, before access of people or animals into the room can be permitted (see Figure 2 below).

The airborne disinfection contact time (ADC) is the time from first release of the product (disinfectant) into the enclosure to the end of the dwell stage (i.e. stages 2+3).

Figure 2: The various phases of a cycle of disinfection of an automatic process



Phases of the cycle:

1: preconditioning (optional)
2: diffusion
3: dwell phase
4: terminal phase (aeration)

Particular attention must be given to the diffusion time and contact time. The diffusion time is the time necessary to reach a target concentration of the product in the air of the room and on the surfaces to be disinfected in a given volume, while the contact time is the time necessary to reach the expected efficacy.

Note: the various phases of the cycle presented are theoretical and can be adapted according to the process. In specific cases, the maintenance of a concentration of biocide in the atmosphere may be achieved by the regular introduction of additional biocide during the contact phase.

5.4.2.5.3 Data requirements

Airborne disinfection differs from the direct application of liquids to surfaces. Therefore the EN phase 2, step 2 standards for surface disinfection are not applicable for room disinfection.

The following test is required for a room disinfectant:

- simulated-use test, such as EN 17272 for disinfection using the airborne application (phase 2, step 2).

EN 17272 test method consists of two parts:

Part 1: Efficacy test, intended to ensure that minimum efficacy requirements are fulfilled for each type of activity claimed and for the targeted application area(s).

Part 2: Distribution test: intended to ensure the efficacy of the process throughout the enclosure. It is performed with a reference test organism at 4 sampling positions.

Every automated airborne disinfection cycle/application is unique, and the purpose of EN 17272 is to provide a defined challenge for the automated airborne disinfection system to successfully meet in order to be considered an efficacious process. The standard method should therefore not be regarded as a validation for all intended treatments with a particular automated airborne disinfection system (see also 5.4.2.5.5 Provisions to be taken into account).

This method is used to qualify the process, i.e. the device(s) and product(s) needed for implementation. For such chemical processes, the combination of the technical characteristics of the device and product cannot be separated.

Principles of room (enclosure) disinfection
Inert and dry carriers contaminated with a known number of micro-organisms (bacteria, fungi, yeasts, mycobacteria, spores and viruses including bacteriophages) are placed in a room of defined volume, temperature and relative humidity. The size of the test room should be relevant to the claims for the product (see below for more information on room size). The carriers used are often stainless steel, but other non-porous relevant materials can also be used, such as glass or plastic.

When the disinfection of textiles (curtains etc.) and other materials (e.g. wallpaper, filters in flow cabinets) is claimed, appropriate carriers should be used to demonstrate efficacy. The standard EN 17272 does not include porous carriers, therefore the test should be adapted for porous surfaces, otherwise different tests should be used. In that case, a justification for the relevance of the tests used should be provided. The test design should be discussed with and agreed upon by the CA before testing takes place. The evaluation will be done on a case-by-case basis by the CA.

Depending on the area of use, suitable interfering substances should be tested (e.g. blood for use in hospitals in dirty conditions). Nevertheless, this test includes also the use of skimmed milk as a protective substance in order to maintain the viability of the sensitive micro-organisms on the carriers during the test.

The inoculated carriers must be placed in a vertical position with inocula facing away from the diffuser. Their distance to the diffuser depends on the room dimensions (for instance: see Annex A of EN 17272). The test method defines obligatory test conditions for parameters that may influence the success of the disinfection (temperature, humidity, volume of the enclosure).

Similar carriers are placed under temperature and relative humidity conditions simulating as close as possible those recorded in the enclosure at the start of the test, to act as controls.

Additional tests can be performed to simulate specific conditions that are encountered in the practice and to fit with label instructions. In this case, all experimental conditions should be described clearly in the test reports.

Automated airborne system:
Disinfection efficacy of automated airborne systems depends on many parameters apart from the biocidal product itself. These parameters are related to the room to be disinfected (e.g. spatial configuration, types of surfaces), ambient conditions (e.g. temperature, humidity) and the device used (e.g. the diffusion principle).

Therefore, a detailed description of the technical characteristics of the equipment and parameters used in efficacy tests must be provided:

- equipment name and model;
- diffusion principle, e.g.:

- nebulisation/cold fogging: generation of an aerosol of discrete particles by any process except heating the biocidal product;
- vaporisation: generation of a vapour from a liquid biocidal product;
- thermal fogging: generation of an aerosol of discrete particles by any process that involves heating the biocidal product;
- fumigation/gassing: with any active substance that naturally is a gas at ambient conditions;

- description of the diffusion performance of the equipment (e.g. maximum room size to disinfect, average flow rate, average droplet diameter (µm rate) where applicable);
- description of the ambient conditions (e.g. relative humidity, temperature);
- diffusion time for a specific volume;
- product concentration (w/w)
- application rate, e.g. ml diluted product/m^3;
- size of the room;
- contact time;
- clean/dirty conditions.

Note, for systems that apply large quantities of product to the surface resulting in run-off from the test carrier, the EN 17272 test must be modified to collect and account for any microorganisms mechanically removed from the carrier.

Contact time
As room disinfection may necessitate a long period of treatment, the contact time to be tested is not defined. The testing should demonstrate efficacy at a contact time proposed for the intended use. This should be relevant to practical use.

Room (enclosure) size
The rooms to be disinfected vary from rather small to quite large. EN 17272 defines the intended use volume claims and required tests as indicated in the table below.

Table 9. Required tests in accordance with the manufacturers intended use volume claims from EN 17272.

	Small enclosures	Large enclosures
Intended use volume	Between 0.25 m^3 and 4.0 m^3	>4 m^3
Required tests	Tests under obligatory conditions in an enclosure between 0.25 m^3 and 4.0 m^3	Tests under obligatory conditions in an enclosure 30 m^3 to 150 m^3
Distribution test	A distribution test should be carried out	A distribution test should be carried out

If a process cannot be tested in accordance with the obligatory conditions due to it being physically unable to operate in an enclosure volume as small as 30 m^3 to 150 m^3, it should be tested in a larger volume as a supplementary obligatory condition.

Test organisms

The test organisms are indicated in the applicable standard methods. Appendix 3 contains a table of reference test organisms.

The general data requirements for PT2 for specific claims and minimal requirements in healthcare also apply for airborne room disinfection.

5.4.2.5.4 Acceptance criteria

A product will be assessed to be sufficiently effective if the required simulated-use test has been performed (using the required test organisms and test conditions), and when the pass criteria for the test have been met.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.2.5.5 Provisions to be taken into account

Limitations

Any limitations of the process should be specified in the application.

Literature has shown that airborne disinfection may not be as effective on wet surfaces (lower concentration of the product) or inside closed cupboards and closets (where the vapour cannot penetrate). Therefore, the SPC should contain appropriate use instructions (such as stating that cupboard doors should be opened, surfaces should be dried and wet areas (such as sinks and toilet bowls) should be disinfected with suitable alternative products), unless the efficacy test demonstrates that efficacy is also ensured under such adverse conditions.

Parameters to be taken into account in the SPC:

It has to be noted that product authorisation should not be limited specifically to the equipment described and used in the application. Nevertheless, the technical characteristics of the described equipment in combination with environmental factors and the properties of the biocidal product are decisive to the outcome of disinfection.

Then following items should be included in the SPC:

- diffusion principle, e.g. nebulisation, fumigation
- range of median droplet diameters (µm), where appliable
- type of surfaces
- contact time
- application rate, e.g. ml/ m^3
- concentration of the product (as used by the user)
- room size (m^3)
- temperature
- humidity recommendation
- pre-cleaning if needed

Biological and chemical validation

As devices cannot be authorised under the BPR, the microbiological and, if possible, chemical validation at the place of use is of crucial importance for these uses. Moreover, other factors that may influence the efficacy of the process in practical use such as the furniture, special structures (e.g. bumps on the walls) or special materials (copper in hydrogen peroxide procedures), including environmental conditions (e.g. temperature, relative humidity) which may affect the success of the disinfection, should also be considered.

Considering these influencing factors, a requirement should be included in the SPC to validate the airborne surface disinfection process is suitable for the room and activities concerned, and that it meets the requirements set in the use instructions for the diffusion regime (dosing application rate, contact time, temperature, humidity, volumes of enclosure, concentration in the air, and contact time during each phase) for specific circumstances of the room (volume, presence of furniture, equipment, cables, etc.).

Therefore, the following sentence concerning mandatory (micro)biological validation should be included in the SPC: *"The user shall always carry out a microbiological validation of the disinfection in the rooms to be disinfected (or in a suitable "standard room", if applicable) with the devices to be used, after which a protocol for disinfection of these rooms can be made and used thereafter."* Biological validation is performed by monitoring efficacy against a challenging test organism (e.g. *Geobacillus stearothermophilus* spores) during the room disinfection process.

In case there are methods available for chemical monitoring of the active substance in the air or on surfaces, it is highly recommended to include in the use instructions in the SPC the advice that besides biological validation, chemical validation should be performed. In case of hydrogen peroxide this can be done with H_2O_2 test strips, or with a device that measures the concentration of H_2O_2 in the air.

5.4.2.6 Swimming pools, spas and hot tubs

5.4.2.6.1 Introduction

Disinfectants are used to treat water in swimming pools, spas and hot tubs. These may be public pools (which may be used by many people daily) or household pools or tubs (which might be used only occasionally). An intermediate situation consists of facilities in hotels, housing complexes or sports clubs, where the bather load may be lower than in a fully public facility, but still high compared to private, domestic facilities.

Disinfectant products can be added to a pool continuously, intermittently, by shock dosing or through generation *in situ*. Large public facilities may have dedicated staff to maintain the pool using automated control systems, whereas smaller pools may be treated using manual methods by janitorial staff. Private pools may be treated by individual householders, supplemented in some cases by professional pool treatment personnel. Disinfection is only one aspect of pool maintenance and other activities, such as ensuring the correct pH and the removal of pollutants by oxidation, flocculation and filtration, are essential to ensure adequate water quality.

The principal purpose of the use of disinfectants is to treat the water to prevent the water-borne transmission of pathogens between pool users. Supplementary purposes are to ensure the aesthetic quality of a pool (by ensuring that algae do not result in turbid water or unsightly and slippery microbial growth on pool surfaces, such as the floor and walls of the pool) and to prevent microbial slime and biofilm formation in pipework and related equipment.

This section only deals with disinfection of the pool water and the pipework and related equipment containing pool water. The disinfection of hard surfaces surrounding the pool is covered in section 5.4.2.3 of this Guidance.

5.4.2.6.2 Data requirements

See PT2 general data requirements.

A detailed, non-exhaustive list of the most relevant applications and test methodology is given in Claims Matrix PT2, table for "Swimming pools": the claims matrices are a set of tables linked to this guidance document (see Appendix 1 for more information).

Test methods

For efficacy testing of pool disinfectants, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a pool disinfectant following a tiered approach:

- a quantitative suspension test (phase 2, step 1);
- simulated-use tests with pool water or a surface test (phase 2, step 2)*;
- and a field trial (phase 3)**;

all simulating practical conditions appropriate to its intended use (temperature, contact time, soiling/bather load, etc.).

* A phase 2, step 2 test may be appropriate in cases where a product has a specific use in surface disinfection. Otherwise, a simulated-use test is appropriate for products intended to disinfect the water in a pool or spa.

** In some cases the field trial can be waived. The OECD guidance document (described below) is based on experience with hypochlorous acid/hypochlorite. Therefore, it is acceptable that for products based on hypochlorous acid/hypochlorite the field trial is waived and only laboratory test data are provided. In some other cases, waiving the phase 3 test can also be justified.

The OECD "Guidance Document for Demonstrating Efficacy of Pool and Spa Disinfectants in Laboratory and Field testing" (OECD Series of Testing and Assessment No 170, version dated 08 October 2012) describes laboratory and field test methods, conditions and criteria needed to demonstrate the efficacy of a pool disinfectant. The protocol for field trials should be agreed upon between the applicant and CA before a field trial is initiated.

For products that are used for specific purposes such as disinfecting pipework, filters and filter media, it may be more appropriate to test using the EN 14885 methods for the disinfection of surfaces in institutional applications.

Test organisms

Besides bacteria and viruses, protozoa can also be of importance in swimming pools. Fungi may pose a health hazard on wet surfaces surrounding the pool and can cause slime build-up in the pipework. OECD guidance lists the organisms that normally should be tested. Although algae and protozoa in pools are in general only a problem when maintenance of the pool is not carried out properly, data against algae and/or protozoa should be provided where claims against these target organisms are made.

5.4.2.6.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests and, where relevant, field trials have been performed (using the

required test organisms and test conditions) and the pass criteria for the tests have been met.

When pass criteria are available in the standard tests these should be met.

The OECD guidance document sets out criteria for laboratory and field trials. However, it may be noted that there is a current OECD project review underway to look at criteria for laboratory and field trials.

Where these criteria are not met, the applicant can provide a justification as to why the product should still be considered acceptable.

5.4.2.7 Toilets

5.4.2.7.1 Introduction

Biocides can be used to disinfect toilet bowl surfaces in diverse environments including hospitals, industry, institutions or households. Toilet bowl biocides are available in a wide variety of forms, such as liquids, foams, powders, gels, pastes and tablets.

These products are often applied via pouring around the inside rim of the toilet bowl surfaces with the area scrubbed after a minimum contact time.

Other products are applied in the toilet permanently. They can be attached over the rim of the toilet bowl, stuck directly onto the side of the toilet bowl, placed directly in the cistern (water reservoir), or attached by other means. These products are normally discharged when the toilet is flushed.

Hard surfaces on the inside of toilets are covered by this section. Surfaces on the outside and toilet seats, lids, etc. are covered by section 5.4.2.3 "hard surfaces" of this Guidance.

The use of biocides in chemical toilets, most commonly found on airplanes, trains, and in portable toilets, is not covered in this section, (see section 5.4.2.13 of this Guidance).

5.4.2.7.2 Data requirements

See PT2 general data requirements in 5.4.0.5 and 5.4.2.2.

A detailed, non-exhaustive list of the most relevant applications and test methodology is given in Claims Matrix PT2, table for "Toilet bowls": the claims matrices are a set of tables linked to this guidance document (see Appendix 1 for more information).

Test methods

For efficacy testing of toilet disinfectants, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a hard surface disinfectant:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative surface test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, contact time, etc.).

Several test methods for quantitative suspension and surface tests are available.

Appendix 2 gives a list of recommended test methods. The following documents are recommended for surface disinfection:

- EN 14885: gives an overview of what EN phase 2, step 1 and step 2 test to use for different uses,

if CEN standards are not relevant or available for the use or organisms claimed, the following documents are recommended if appropriately reflecting the application:

- OECD guidance for the testing of biocides: Quantitative method for evaluating the activity of microbicides used on hard non-porous surfaces (these are surface tests which would be considered phase 2, step 2 tests).

The use of the specified tests is strongly recommended where they are relevant and appropriate. Where tests are not appropriate to the product other tests can be used, although a justification for the relevance of the tests used should also be provided.

For products intended to be added to the water reservoir or hanging down from the rim of the bowl, the concentration of the product (or at least the active substance) in the water before, between and after flushing should be determined. This can be done by an analysis of the water under in-use conditions or, for products where all parameters are defined, by calculation. The laboratory efficacy tests should be performed using these concentrations.

Tests in phase 3 are optional.

When efficacy against biofilm is claimed a simulated-use test or field trial has to be provided, next to a phase 2, step 1 test. See section 5.4.2.11 of this Guidance for test methods.

Test organisms

The same test organisms as for hard surfaces should be tested. See section 5.4.2.3.2 of this Guidance and Appendix 3.

Products will normally only target bacteria and, optionally, yeasts and viruses. Fungi and spores are usually not relevant in the toilet bowl but when efficacy is claimed testing is required.

5.4.2.7.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests, these should be met.

If the test does not provide these criteria, the general criteria referenced in Appendix 4 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.2.8 Air-conditioning systems

5.4.2.8.1 Introduction

Disinfection of air-conditioning systems is similar to hard surface disinfection since only the surfaces in the system are disinfected and not the air itself. The difference with general surface disinfection is that the surfaces are mostly hidden inside the system and cannot be reached easily without taking it apart (for instance for air-conditioning systems in cars, dismantling the system would not be desirable).

In general, disinfectants for air-conditioning systems are applied by airborne diffusion of an aerosol, a smoke, a vapour or a gas. The biocide is commonly applied to an air-conditioning system at the inlet of the system. This way the biocide is sucked into and passes through the system when it is operational.

Preservation of cooling liquids is not covered under PT2 but rather within PT11 (preservatives for liquid cooling and processing systems).

5.4.2.8.2 Data requirements

For products that are applied by airborne diffusion of an aerosol, a smoke, a vapour or a gas the same test methods and test organisms should be used as for room/airborne disinfection. Therefore, the same data requirements as for section 5.4.2.5 of this Guidance (Room disinfection/automated airborne disinfection of surfaces) are applicable here.

The following test is required for a disinfectant for air-conditioning systems:

- simulated-use test, such as EN 17272 for disinfection using the airborne application (phase 2, step 2).

See section 5.4.2.5 of this Guidance for specifications.

In the simulated-use test, the carriers inoculated with test organisms are placed in the air-conditioning system at the beginning and at the end of the system. When it is not possible to put carriers in the system, they should be placed between the biocide application site and the inlet of the system and at the end of the system, in the out-flowing air. If carriers at both sides fulfil the criteria it can be assumed that the surfaces in between are also disinfected sufficiently.

For products that are applied by manual spray, the test methods and test organisms used for hard surface disinfection should be employed. See section 5.4.2.3 of this Guidance (Hard surface disinfection) for data requirements.

In addition to these data, the applicant should provide a justification that the spray apparatus is capable of reaching all (hidden) surfaces of the air-conditioning system.

5.4.2.8.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

The same pass criteria can be used as for other surface disinfection (section 5.4.2.3.3 of this Guidance). The criteria referenced in Appendix 4 can be used as guidance for what level of lg reduction is normally required. Deviations from these are possible but have to be justified in the application.

5.4.2.9 Equipment disinfection by immersion

5.4.2.9.1 Introduction

Although instrument or equipment disinfection can be considered equal to hard surface disinfection, it differs from the intended use in section 5.4.2.3 of this Guidance because it is mainly applied by immersion of the equipment or instruments in the biocide solution or by filling the equipment with the solution (disinfection of inner surfaces). The products are intended for equipment used, for example, in healthcare facilities, laboratories and industry. The requirements for products to be used for CIP are not included in this section and can be found in section 5.4.4.3 of this Guidance.

Some of the products used for the disinfection of medical instruments, which are to be used specifically for diagnostic and/or therapeutic purposes for human beings, do not fall under the scope of the BPR. Disinfectants that are specifically used for the disinfection of medical devices or a group of medical devices (anaesthetic equipment, endoscopes, surgical instruments, incubators) are covered under the Medical Device Directive 93/42/EEC.

However, some disinfectants have a broader claim, for example, disinfection of instruments and surfaces. They are so called 'dual-use products' as their distinct claims are covered by more than one legislative instrument. The BPR states that such biocidal products should comply, in addition to the requirements laid down in the BPR with the relevant essential requirements set out in Annex I to Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices, Council Directive 93/42/EEC of 14 June 1993 concerning medical devices and Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices.

5.4.2.9.2 Data requirements

Test methods

For efficacy testing of equipment disinfectants, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for an instrument disinfectant:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative carrier test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Methods for testing the efficacy of equipment or instrument disinfectants are available.

Appendix 2 gives a list of recommended test methods. The following document is recommended for instrument disinfection:

- EN 14885: gives an overview of which EN phase 2, step 1 and step 2 test to use for different uses.

The use of the specified tests is strongly recommended where they are relevant and appropriate.

For disinfection by immersion, the information should be provided on how long the efficacy of the biocide solution can be guaranteed.

For use in industrial and institutional areas, no specific tests for instrument disinfection are given in EN 14885. Nevertheless, phase 2, step 1 suspension tests from the industry and institutional areas can be used, by employing area specific soiling. For phase 2, step 2 tests, the instrument tests for medical areas are most appropriate. Soiling specific to the area of intended use should be employed.

Test organisms

For general disinfection of medical (including dental and veterinary) equipment, instruments, and equipment and other instruments which are used in contact with skin or mucous membranes (e.g. instruments for pedicure), efficacy against bacteria, yeasts and viruses must be demonstrated. For instruments and equipment used in laboratory and industry, the test organisms specified for hard surfaces should be tested.

See section 5.4.2.3.2 of this Guidance, Appendix 3, and Appendix 4.

5.4.2.9.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests, these should be met.

If the test does not provide these criteria, the general criteria referenced in Appendix 4 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.2.10 Textile/laundry process disinfection

5.4.2.10.1 Introduction

Biocides can be used to treat textiles and fabrics in hospitals, healthcare facilities, industry, institutions or private homes, when relevant micro-organisms (pathogenic, spoiling) in the textiles have to be reduced. These products can be in the form of laundry products which combine detergent and biocide or can be specialised products. Specialised products can be in the form of laundry additives that are added to the wash cycle or as finishing products (e.g. fabric softeners) or similarly added as a pre-treatment or to the last rinsing step.

Typically contaminated clothes, linen or other washable textiles are treated in an appropriate washing machine. The biocide is added in concentrated form and diluted in the machine with water according to the specification of the manufacturer to get a defined concentration in the machine. The automated chemical-thermal process normally comprises of an (optional) initial pre-treatment step for heavily soiled laundry, followed by the main washing step (at a defined temperature and defined contact time) and 3 to 4 rinsing steps with cold water.

In some cases, laundry can be treated through a hand-wash process in a diluted biocide, which can take the form of a pre-soak (after which machine washing is used), a hand wash only, or through soaking to disinfect textiles before they are destroyed (e.g. in an infectious disease outbreak situation).

5.4.2.10.2 Data requirements

See PT2 general data requirements. A detailed, non-exhaustive list of the most relevant applications and test methodology is given in Claims Matrix PT2, table for "Laundry products": the claims matrices are a set of tables linked to this guidance document (see Appendix 1 for more information).

Test methods

For efficacy testing of textile disinfectants, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a textile disinfectant:

- a quantitative suspension test (phase 2, step 1),
- a quantitative carrier test involving carriers made of test fabric (cotton, polyester, etc.) (phase 2, step 2),

Both should simulate practical conditions relevant to its intended use (concentration of the product, temperature, soiling, different fabrics, contact time, etc.).

Currently, the following types of test are available:

- phase 2, step 1 suspension tests as described in EN 14885,

- phase 2, step 2 tests involving:
  - a full-scale laundry machine test (EN 16616)
  - for products not intended to be used in washing machines, small-scale laboratory setting (e.g. for pre-soaking in a bucket) may be considered (e.g. ASTM E2406 or ASTM E2274).

In phase 2, step 2 tests fabric is contaminated with test organisms and then exposed to the disinfectant.

The EN tests are strongly recommended where available and appropriate.

For biocidal products used as disinfectants in combination with detergents, e.g. in the pre- and main-wash, the following approach should apply:

- Phase 2, step 1 test should be done in combination with the detergent and disinfectant. All claimed disinfectant/detergent combinations and the claimed conditions should be tested unless worst-case conditions can be justified (e.g. testing only lowest and highest concentrations of same disinfectant/detergent combination).
- Phase 2, step 2 test should be done according to EN 16616. Furthermore, as a minimum, the disinfectant/detergent combination should be tested. In principle all claimed disinfectant/detergent combinations and the various conditions claimed should be tested, unless worst-case conditions can be justified (e.g. testing only lowest and highest concentrations of same disinfectant/detergent combination).

For biocidal products used as disinfectants and applied separately without a detergent, e.g. disinfection in the last rinse for textile, the following approach should apply:

- Phase 2 step 1 test should be performed without a detergent.
- In case a disinfectant is applied in such way that it does not come into contact with a detergent, a justified suitable test procedure for the phase 2, step 2 test should be provided, e.g. a modified EN 16616 test without detergent, with justification for the use of soiling that mimics the clean conditions.

For combined cleaner-disinfection products used as disinfectants for textile, the following approach should apply:

Phase 2, step 1 and phase 2, step 2 tests should be done with the combined cleaner-disinfection product (without adding an extra detergent since the detergent is already included in the product).

Table 10. Efficacy testing versus disinfection at various steps of the washing process.

Disinfection in	Presence detergent / disinfectant in washing step	Testing	Test conditions
Pre-wash*	detergent and disinfectant	Phase 2, step 1 Phase 2, step 2	Detergent and disinfectant at use concentration Temperature and contact time as in-use instructions Dirty conditions
Main wash*	detergent and	Phase 2, step 1	Detergent and disinfectant at use

	disinfectant	Phase 2, step 2	concentration Temperature and contact time as in-use instructions Dirty conditions
Last rinse*	Disinfectant	Phase 2, step 1 Phase 2, step 2	Temperature and contact time as in-use instructions Clean conditions**

* Steps of the assumed washing cycle are: 1) pre-wash, 2) main wash, 3) rinse.

** EN 16616 describes dirty conditions only. When clean conditions are in line with the intended use justified modifications can be made to the interfering substance specified for loading the washing machine in EN 16616.

Test organisms

Textile disinfection products should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided. For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

Depending on the intended claims, the following test organisms can be chosen to be tested at the claimed temperature.

Table 11. The recommended test organisms for efficacy testing of textile disinfection processes.

Claimed temperature (T)	Test organisms
T ≤ 40ºC	Organisms as indicated in EN 16616 (viruses as indicated in phase 2, step 1 test)
40°C < T < 60ºC	*Pseudomonas aeruginosa* *Escherichia coli* (K12) *Staphylococcus aureus* *Enterococcus faecium* *Candida albicans* *Aspergillus brasiliensis* *Murine Parvovirus* *Mycobacterium terrae* *Mycobacterium avium* All claimed groups of target organisms need to be tested (phase 2, step 1 and phase 2, step 2). Additional water control at 20°C is needed to validate the organisms, and to show that test conditions not related to temperature have no adverse effect, as indicated in EN 14885.
T ≥ 60ºC	*Enterococcus faecium* *Murine Parvovirus* Valid tests (phase 2, step 1 and phase 2, step 2) against *E. faecium* permit claims against bacteria, yeasts, fungi and mycobacteria.

An overview of reference test organisms, also for high temperatures, is given in Appendix 3.

Test conditions

For products intended to be added to washing machines, information on the following in-use conditions should be provided:

- the concentration of the product in the water during disinfecting process (i.e. washing or rinsing). The water volume used can differ between wash and rinse cycle and different washing programmes, but also between washing machines;
- the water to the textile ratio in the test is an important factor that should reflect the in-use conditions;
- the temperature during the disinfection process (high when added in wash process, low in rinse process);
- the contact time (differs between various washing programmes and washing machines).

The laboratory tests should be performed under these conditions. The conditions for effective disinfection can normally only be carried out in professional washing machines.

If the exact conditions cannot be met, for example, in household machines, reasonable worst-case conditions must be tested.

Worst-case conditions, e.g.:

- the lowest temperature;
- the highest volume of water (i.e. maximum dilution of the product);
- the shortest contact time;
- the maximum load of laundry (i.e. smallest water to textile ratio).

When phase 2, step 2 tests involving fabric test carriers are performed, both the micro-organisms remaining on the test carriers, those released into the washing liquid and those transferred to previously uncontaminated control carriers should be assessed.

Manual soaking or pre-soaking can be done at room temperature but for some intended uses the temperature might start high and will cool down during the contact time (e.g. where hot water is used, which cools naturally). This should also be taken into account in the tests.

Soiling

The interfering substance most appropriate for the in-use conditions should be used.

5.4.2.10.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met. When the product is intended to be used in combination with or directly after a detergent, a clear effect of the disinfectant alone should be demonstrated. There should be a significant difference (+lg 2) between disinfectant+ detergent and the detergent alone.

EN and VAH tests provide pass criteria.

No acceptance criteria have been specified in the ASTM standards for laundry (ASTM E 2406-04 or ASTM E 2274-09).

If the test does not provide pass criteria, the general criteria referenced in Appendix 4 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.2.11 Biofilms

5.4.2.11.1 Introduction

A biofilm is a complex aggregation of micro-organisms usually distinguished by the excretion of a protective and adhesive matrix attached to a solid surface in contact with a fluid. The matrix may incorporate other components derived from the environment.

Once the first cell succeeds in attaching to a surface and a biofilm starts to form, growth of the biofilm may become very fast, as subsequent free floating bacteria find it much easier to attach to the developing matrix.

Biofilms can grow in areas such as inside water tanks and the distribution pipelines of hospitals, hotels, industries and, in general, in any water systems which have temperatures and nutrients adequate for microbial growth.

The consequences of biofilm formation in a water system or facility may be severe depending on environmental conditions and any safety and performance requirements.

In healthcare facilities, biofilm contamination of medical equipment or water systems may increase the risk of nosocomial infections; in industrial facilities, biofilm may cause microbial contamination of production (pharmaceuticals, cosmetics, etc.); in other situations, biofilms may be responsible for significant reduction of the performance of water systems by obstructing normal flow or they may induce corrosion of the pipelines.

Several factors may contribute to biofilm formation, with important factors including the chemical composition and roughness characteristics of the pipe, tank or tube circuit.

Bacteria in biofilms are more resistant to disinfection than planktonic bacteria of the same species, as the presence of extracellular polymeric substances can act as a physical barrier to the biocide. This matrix may hamper biocidal penetration to the lower layers of the biofilm or may interact with the biocide and neutralise it. Additionally, the physiological state of the bacteria in the biofilm differs from bacteria in suspension, which can also influence the susceptibility of the bacteria to biocides. Complex communication systems are often also present that allow increased tolerance of members of the biofilm community to be initiated.

Two types of activities of biocides against biofilm can be identified:

1) Prevention of biofilm formation: the biocide acts on biofilm formation (i.e. in this case the biocide is present before the biofilm is formed and may affect the early adhesion of cells to the surface or the viability of the cells);
2) Biofilm disinfection ("curative"): the biocide acts on a mature biofilm (i.e. when the biofilm is already present on a surface and the biocide interacts with the biofilm-embedded cells, with a -cidal effect). Biocidal products of this type may also achieve detachment of the biofilm (possibly in conjunction with physical action).

In case where the biofilm is not removed as a result of the biocide treatment, it should be followed by the mechanical removal of the biofilm.

The industry is increasingly developing new technologies for prevention, inactivation and/or detachment of biofilms and/or inactivation of biofilm embedded organisms, for example through the use of UV light, water ionization or impregnated or coated materials and new biocides which claim specific efficacy against biofilms.

5.4.2.11.2 Data requirements

There are currently no standard laboratory tests available to verify the efficacy of biocides against biofilms. As this is an area in which science is developing rapidly, the information

below should be considered as general guidance reflecting the state of knowledge at the time of writing this Guidance.

Tests to demonstrate the efficacy of disinfectants according to EN and OECD are based on simpler models than are found in biofilms. The available surface/carrier tests are not representative of biofilm models, as they do not consider the presence of extra cellular polymeric substances which act as a physical barrier to the biocide.

Other characteristics of the biofilm and biocidal product should be taken into account. For example, if biocide impregnated materials claim a preventive effect on biofilm formation, the prevention of biofilm formation should be demonstrated, taking into consideration the half-life of the impregnating substance which may differ depending on the material characteristics. The active substance may be released from the surface and/or may be inactivated by environmental factors.

A standard suspension test can only be used to confirm the basic activity of the product against the claimed organisms in a tiered approach.

A suggested general approach could be:

1) a suspension test: any biocide claiming to act on biofilm, has to be first evaluated in standard suspension test (preferably EN);
2) a simulated-use efficacy test to demonstrate the ability of the product to exert a controlling effect on the biofilm under either static conditions or under flow conditions depending on the use pattern (claim). This controlling effect can be to destroy and detach, inhibit or prevent the formation of a biofilm;
3) a field trial, where the biofilm is formed under (simulated) use conditions.

These tests should be performed in sequence to obtain more complete information on the activity of the product on biofilm.

For biofilm disinfection (curative) a suspension test (as for (1) above) and suitable robust data from either a simulated-use test (2) or field trial (3) should be performed. If there are no robust data from a simulated-use test (2), a field trial (3) is mandatory.

For biofilm prevention, the approach is different to that for biofilm disinfection, as the biocide is present before the biofilm is formed and may affect the early adhesion of cells to the surface or the viability of the cells. In this case, the suspension test (1) may not be useful since the product might not have a cidal effect.

Test methods

Suspension tests
The first step in the tiered approach is a suspension test. The CEN phase 2, step 1 tests are suitable as suspension tests. This test is only applicable for products that can be tested in suspension and which have a cidal effect.

Simulated-use tests
Standard laboratory tests to verify the efficacy of biocides against biofilms are not currently available. Therefore, before performing a biofilm test, the methods should be agreed upon with the CA.

Applicants should provide a method following the principles in this guidance and based on scientific evidence. During the development of the tests, CAs should be consulted to make sure that the tests are acceptable.

Biofilms can be formed and evaluated in static or flow conditions. The way the biofilm is formed has an effect on the susceptibility of the biofilm to biocides: biofilms formed under flow conditions are generally more resistant to biocides than biofilms formed under static conditions.

The conditions under which the biocidal products will have to operate should also be taken into account. Under static conditions, the disinfectant operates without the aid of the removal effect of a fluid flow or shear stress. Under flow conditions the contact time might be shorter when shock dosing is used.

Static tests are less expensive and easier to standardise, but flow tests are generally closer to the real use scenarios.

In both cases, the reproducibility and repeatability of results over time should be ensured; so a method that allows a series of observations, rather than a single observation, should be employed.

Laboratory tests for evaluating the efficacy of biofilm disinfectants should emulate the critical factors of a real-world environment. In most instances, a biofilm will not be comprised of a single species and tests based on consortia relevant to the end use should be employed when simulating actual use.

In cases where only efficacy against biofilms formed under static conditions is claimed (e.g. use in tanks without flow) it is sufficient to only test against these biofilms.

Examples of methods for testing under flow and static conditions are described below, but other protocols are available in the literature or may be under development.

Static condition assay

Standard laboratory tests to verify the efficacy of biocides against biofilms formed under static conditions are not currently available. However, the literature describes several methods of how to create a biofilm in the laboratory under static conditions.

An example of a semi-quantitative method for biofilm evaluation is the microplate test, where a biofilm is formed in static conditions and the amount of biofilm can be quantified by spectrophotometric measurements. The number of living cells in the biofilm before and after treatment can also be determined. In this case, the disinfectant operates without the aid of the removal effect of a fluid flow or shear stress.

A positive aspect of such an assay is that it is a low cost, easy-to-conduct test, that allows several replicates and/or the testing of several conditions (several biocide concentrations, more species, etc.) to be performed, which would provide the basis for a more accurate and closer-to-reality test.

This method consists of the formation of a biofilm by the species of interest on the bottom of 96-well plates (the material and coating of the plates should be specified); the disinfectant may be present before (preventive effect) or after (inhibition/removal effect) the biofilm is formed. The amount of biofilm (biomass) is quantified after staining of the adherent material and spectrophotometric measurement. Detecting chemicals such as ATP to measure bacterial viability may also be used.

Flow condition assay

Standard laboratory tests to verify the efficacy of biocides against biofilm formed under flow conditions are not currently available. However, systems to generate a standard biofilm have been developed by CEN (CEN ISO/TS 15883-5:2005 Annex F) and ASTM (ASTM E2196 and ASTM E2562). Using either of these reproducible biofilms, a method for the assessment of

prevention and/or elimination of biofilm in terms of viable cells reduction and bacterial biomass reduction can be carried out.

The CEN method consists of the production of a standard *Pseudomonas aeruginosa* biofilm inside a Teflon tube, using a flowing system to simulate a real world situation.

ASTM E2196 and ASTM E2562 standards use biofilm rotating disc reactors, which are especially suited for high shear forces.

The biofilm is then treated with a disinfectant to evaluate the biocidal capacity to remove or to reduce the biofilm.

Other carrier types (e.g. silicon, steel, PVC, etc.) can be selected and used depending on the biofilm development system, and the experimental conditions can be adapted to compare the efficacy of different treatments in preventing biofilm formation.

A reference substance of known activity must be tested in parallel (e.g. chlorine dioxide, sodium hypochlorite).

Field trials

As for other situations in which biocides are used, only field trials (phase 3 tests) are fully representative of the activity of the biocide on biofilms, but these tests are difficult to standardise, and such tests should be complemented by laboratory suspension or simulated-use tests, which have a higher degree of robustness and reproducibility.

A field trial should reproduce the in-use conditions of the worst-case situation of the intended uses.

Prevention and/or elimination of biofilm (in terms of viable cells reduction and bacterial biomass reduction) should be demonstrated by sampling before and after disinfection.

A field trial can be waived if a suitably robust simulated-use test, which adequately mimics the in-use conditions is provided. A robust test could for instance be a complex pipe system, in which natural biofilm formation takes place, either in combination with the addition of standard organisms or not.

Test organisms

The choice of micro-organisms for a test is relevant since the use of only one organism per test is limiting and may not be fully representative of the real events leading to micro-organism aggregation (biofilms in settings where disinfectants are used, are normally multi-microbial, i.e. composed of several different species). Moreover, contaminants from environmental sources may be embedded in the biofilm matrix which may reduce the disinfectant's efficacy.

Bacteria are not the only inhabitants of biofilms, as both fungi and algae may also inhibit biofilms. Protozoans that consume bacteria may feed on biofilms. Protozoan oocysts and virus particles can become entrapped in a biofilm and later detach, returning to the environment.

In a suspension test, the standard organisms per claimed group (bacteria, fungi, etc.) should be tested.

For a general claim of efficacy against biofilms, as a minimum, bacteria should be tested in laboratory biofilm tests. When action against other groups of organisms (e.g. fungi, algae, etc.) is claimed these should be tested as well.

In suspension tests, the standard organisms should be tested (see Appendix 3).

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Legionella* spp. are acceptable test organisms for laboratory biofilm tests. Mixtures of test organisms for producing biofilms are only acceptable as additional tests as it is difficult to standardise such methods.

In simulated-use tests or field trials the biofilm may be formed *in vivo* with naturally occurring micro-organisms.

5.4.2.11.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, where relevant, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests, these should be met.

If the test does not provide these criteria, the general criteria referenced in Appendix 4 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.2.12 Soil

Disinfection of soil and other substrates (in playgrounds) with biocides is not common (and so far not claimed for Annex I of the BPD or the "Union list of approved substances" of the BPR). This is more often done for plant protection. Therefore, plant protection guidelines and EPPO standards on soil treatments should be referred to for test methods. The use of the test methods should be justified with the application.

5.4.2.13 Other uses in PT2

Several other uses are mentioned in the description of PT2: wastewater and hospital waste disinfection, algaecides for swimming pools and indoor/outdoor aquatic area (aquaria/garden ponds), foot baths in swimming pools, chemical toilets, disinfection of air. No data requirements and acceptance criteria for these uses are currently available.

However, the general principles for efficacy evaluation in PT2 are applicable for these other uses. Efficacy data should be adequate to demonstrate efficacy and suitability for the intended use, based on laboratory and/or practical data from existing and/or proposed new quantitative studies. If desired the design of any proposed efficacy tests may be agreed between the applicant and the CA taking into account all relevant conditions of use. Such factors include consideration of the organisms to be controlled, requirements for biocidal or biostatic effects, contact time and temperature and the nature and presence of interfering substances.

Specific requirements should also be set on a case-by-case basis by the CA as appropriate for specific claims.

5.4.3 PT3 Veterinary hygiene biocidal products

5.4.3.1 Introduction

Product Type 3 contains biocidal products used for veterinary hygiene purposes such as disinfectants, disinfecting soaps, oral or corporal hygiene products or with anti-microbial function. Products used to disinfect the materials and surfaces associated with the housing or transportation of animals are also included.

Some of the products in PT3 are on the borderline with veterinary medicinal products or cosmetic products. If the product under investigation is within the scope of the Veterinary Medicinal Products Directive (2001/82/EC as amended by 2004/28/EC) it is excluded from

the BPR for the respective use. When a product only has a cosmetic claim, e.g. cleaning skin, hoofs, paws) and no reference is made to any biocidal claim, e.g. skin disinfection, activity against micro-organisms, it is excluded from the BPR.

Borderline cases are discussed in more detail in the respective sections below. Regarding disinfectants used in veterinary practices and hospitals see the agreement reached at the CA meeting in May 2015 (CA-May -2015-Doc 8.3 - final)[20].

In the sections below the requirements and acceptance criteria for most common uses are specified. For other uses and claims that are not specifically mentioned the requirements will be set on a case-by-case basis by the CAs.

5.4.3.2 Disinfectants for hard surfaces in PT3

5.4.3.2.1 Introduction

Biocides can be used to disinfect hard surfaces, both porous and non-porous, in areas such as animal housing (stables, cages, housing for pets, etc.), animal transportation vehicles (including tyres), hatcheries, etc. These surfaces may be tables, floors, walls, the outsides of (milking) machinery (including milking robots and milking clusters/claws) and hard furniture, equipment, boots, etc. Products may be applied by spraying, wiping, foaming or soaking, and may be washed or wiped off after a certain contact time. Boots and tyres may be disinfected by walk-through, drive-through bath or mat, or even by a machine (boot wash station), etc.

The testing requirements for some specific uses of hard surface disinfectants are discussed in separate sections, for example, beehives.

Disinfection of inner surfaces of pipelines or reservoirs for milk, water or feed for animals are considered food and feed contact surfaces and are therefore considered PT4 (see section 5.4.4.3 of this Guidance). Outer surfaces of milking equipment are considered in this section.

5.4.3.2.2 Data requirements

Test methods

For efficacy testing of veterinary hard surface biocidal products, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a hard surface disinfectant:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative surface test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, porous or non-porous surfaces, contact time, etc.).

Field trials in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

Several methods for testing the efficacy of hard surface disinfectants are available.

Appendices 2 and 4 give a list of recommended test methods.

The following documents are recommended for surface disinfection:

[20] CA-May15-Doc.8.3 - final - Disinfectants in veterinary practices and hospitals

- EN 14885: gives an overview of which EN phase 2, step 1 and step 2 tests to use for different uses;
- DVG guidelines: relevant for testing against endoparasites and yeasticidal, fungicidal, virucidal and mycobactericidal activity on porous and non-porous surfaces, as long as no EN tests are available (available at https://www.desinfektion-dvg.de/index.php?id=2219).

The use of the specified tests is strongly recommended where they are relevant and appropriate. Where the tests are not appropriate to the product, other tests can be used, although a justification for the relevance of the tests used should also be provided.

Since OECD tests are not specified for veterinary use, they are not specifically recommended.

In the veterinary area very often rough, porous surfaces have to be disinfected (i.e. wood, concrete, rough plastic materials). When tests for porous surfaces are available it is recommended to use these tests for general surface disinfection in veterinary areas.

For boot, tyres, and equipment disinfection by immersion in a bath, the information should be provided on how long the efficacy of a bath can be guaranteed (time period, number of boots, etc. passing through). Challenging efficacy tests, e.g. capacity tests, see section 5.4.0.4.1 of this guidance, should be done simulating the consecutive challenge not only by micro-organisms but also by soiling. A test with relevant organic soiling should be provided in order to ensure that the biocidal product can be challenged successfully with the test organism until the end of the claimed period of use. Alternatively, for products with one active substance that can easily be measured, efficacy can be demonstrated using a field trial in which the amount of active substance is measured several times during the test period. Efficacy (suspension) tests should be provided with the concentration of the product tested (in the suspension test) and the active substance concentration obtained in the field at the end of the claimed period of use.

When efficacy against a biofilm is claimed, a simulated-use test or field trial has to be performed, along with a phase 2, step 1 test. See section 5.4.2.11 of this Guidance for test methods.

Where no phase 2, step 2, or phase 3 tests are provided this must be justified in the application for authorisation and will be evaluated on a case-by-case basis.

The EN tests are strongly recommended where available and appropriate. For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Relevant groups of organisms to be controlled in the veterinary area can be bacteria, yeasts, fungal spores, viruses, mycobacteria, bacterial spores, and endoparasites (oocysts).

Veterinary hard surface biocidal products should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

Products for disinfection of veterinary instruments and/or animal transportation vehicles should not only be effective against bacteria and yeasts but also against viruses.

Activity against fungi is also required for products used in hatcheries.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference test organisms is given in Appendix 3.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC.

The claimed contact time has to be a realistic value, for instance:

- for surface disinfection products used on the outside of animal transport vehicles (specifically tyres) the contact time should not exceed 5 minutes;
- for disinfectants used on boots applied by spraying or walk-through bath the contact time should not exceed 1 minute;
- for disinfectants applied by dipping in the bath, used on boots, materials, etc. the contact time should be as claimed in the SPC;
- for surface disinfection products used in animal housing on floors, walls, etc. the contact times as stated in the standard tests should be taken into account.

Additional contact times can be considered if appropriate and justified by the application (e.g. overnight disinfection).

Tests should be carried out with soiling for clean or dirty conditions (low- or high-level soiling) in accordance with the test requirements. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning prior to disinfection is necessary. If this is not stated in the SPC, the test should be done under dirty conditions. The soiling needed for clean and dirty conditions can be found in the relevant EN tests and are referenced in Appendix 4. When the test does not state two levels of soiling the soiling referenced in Appendix 4 should be used.

Normally PT3 products are tested at 10ºC or below since the temperature in animal housings may be low. For some uses higher temperatures are acceptable (e.g. hatcheries). Deviations from this temperature requirement must be justified in the application and will be evaluated on a case-by-case basis. Any limitations on the temperatures at which the product should be used, and for which efficacy has been proven should be stated in the SPC.

5.4.3.2.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.3 Disinfection of beehives and beekeeping equipment

5.4.3.3.1 Introduction

Disinfection of beehives is done to prevent spread of diseases from one bee population to the next.

Only disinfection of empty beehives and beekeeping equipment, with products without a medicinal claim, is a biocidal use for general disinfection. Products used in beehives with bees, honey and brood combes are veterinary medicinal products. These products are within

the scope of the Veterinary Medicinal Products Directive (2001/82/EC as amended by 2004/28/EC) and are therefore excluded from the BPR.

Important disease which can be spread via beehives are American foulbrood (*Paenibacillus larvae*), European foulbrood, (*Melissococcus plutonius*), Nosema (*Nosema apis, Nosema ceranae*), chalkbrood (*Ascosphaera apis*) stonebrood (*Aspergillus flavus*) and some viral diseases. Of these diseases American foulbrood, which is an endospore-forming bacterium, is the most difficult to control.

Normal practice in case of American and European foulbrood is to clean/disinfect beehives and beekeeping equipment and additionally disinfected by scorching with a blowtorch.

5.4.3.3.2 Data requirements

Test methods

For efficacy testing of disinfection products for beekeeping, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for disinfectants for beehives:

- a quantitative suspension test (phase 2, step 1;
- and a quantitative carrier test (phase 2, step 2) for porous surfaces;

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Field trials in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

There are no standard tests available specifically for use in beehives. Phase 2, step 1 EN tests for the veterinary area are suitable, and for sporicidal activity the EN 13704. EN phase 2, step 2 tests for the veterinary area on porous material would be suitable but they are not available for all organisms yet. This can be either EN 16437 phase 2, step 2 test on bacteria for the veterinary area on porous material or DVG guidelines on rough surfaces. These tests can be adapted for other organisms.

In these tests, a reference substance must be included.

Where no phase 2, step 2 tests for the veterinary area on porous material are available, the available test should be adapted for this use (e.g. EN 16437 adapted for other organisms).

When the claim for the product is to replace both the cleaning/disinfection step and the flaming with a welding torch, a field trial has to be provided in which it is demonstrated that the product is as efficacious against foulbrood infected hives as is cleaning with sodium hydroxide combined with scorching with a blowtorch.

For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Disinfection products for beehives should be at least sufficiently effective against bacteria and bacterial spores. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

For bacterial spores, only a test for the food area is available (EN 13704). For disinfection products for beehives spores of two bacterial species should be tested. Next to the current standard test organism, *Bacillus subtilis* spores, also *Bacillus cereus* should be tested.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC.

The claimed contact time has to be a realistic value.

It must be ensured that the disinfected parts stay wet during the contact time. When residual efficacy is claimed for dried products, this should be demonstrated in efficacy tests.

For disinfection of beehives and beekeeping equipment, tests should be performed under dirty conditions (high-level soiling) used for surfaces in the veterinary area. If beehives are not cleaned before disinfection the high-level soiling for suspension tests should be used, also in the porous surface test and tests adopted from other areas of use (e.g. EN 13704).

The soiling needed for dirty conditions can be found in the relevant EN tests and referenced in Appendix 4.

For disinfection of beehives a temperature of 10ºC or lower is acceptable. Deviations from this temperature requirement must be justified in the application and will be evaluated on a case-by-case basis.

5.4.3.3.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.4 Animal feet disinfection

5.4.3.4.1 Introduction

Animal feet disinfection includes hoof and claw disinfection. Products are applied in a bath, through which the animals can walk, or as wipes, foam, spray, etc. See section 5.4.3.1 of this Guidance for overlap with other EU directives.

5.4.3.4.2 Data requirements

Test methods

For efficacy testing of animal feet disinfection products, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for an animal feet disinfectant:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative carrier test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Field trials in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

There are no standard tests available specifically for use on animal feet. Phase 2, step 1 EN tests for veterinary area are suitable. Since hoofs are made of porous material EN phase 2, step 2 tests for the veterinary area on porous material would be suitable but these are not

available for all organisms yet. Alternatively, DVG guideline tests on rough surfaces can be used.

The phase 2, step 2 test design must always reflect the application. When no standard test is used the test design should be discussed with, and agreed upon by, the CA before testing takes place.

When no phase 2, step 2, or phase 3 tests are provided this must be justified in the application and will be evaluated on a case-by-case basis.

For disinfection in a hoof bath, the information should be provided on how long the efficacy of a hoof bath can be guaranteed (time period, number of animals passing through). Challenging efficacy tests, e.g. capacity tests, see section 5.4.0.4.1 of this Guidance, should be done simulating the consecutive challenge not only by micro-organisms but also by soiling. A test with relevant organic soiling should be provided in order to ensure that the biocidal product can be challenged successfully with the test organism until the end of the claimed period of use. When a challenge test is provided and the efficacy of the challenged solution has been determined at the end of the challenged period the quantitative suspension test can be waived. Alternatively, for products with one active substance that can easily be measured, efficacy can be demonstrated using a field trial in which the amount of active substance is measured several times during the test period. Efficacy (suspension) tests should be provided with the concentration of the product tested (in the suspension test) and the active substance concentration obtained in the field at the end of the claimed period of use.

For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Animal feet disinfection should be at least sufficiently effective against bacteria. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC.

The claimed contact time has to be a realistic value, therefore maximum contact times are set.

For animal feet disinfection products the contact time should not exceed 5 minutes.

It must be ensured that it is possible to keep the disinfected parts wet during the contact time in practice. When residual efficacy is claimed for dried products, this should be demonstrated in efficacy tests.

Tests should be carried out with high-level soiling conditions in accordance with the test requirements. Soiling conditions for animal feet disinfectants are the same as for other veterinary area disinfectants. The soiling needed for dirty conditions can be found in the relevant EN tests and referenced in Appendix 4.

Normally animal feet disinfection products are tested at 10ºC since feet disinfection is often carried out outside animal housings at low temperatures. Deviations from this temperature requirement must be justified in the application and will be evaluated on a case-by-case basis.

5.4.3.4.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.5 Teat disinfection

5.4.3.5.1 Introduction

Teat disinfection products are used to disinfect the teats of the udder of dairy animals, e.g. cows, sheep and goats, before or after milking. Products can be applied by dipping, spraying, foaming, wiping, etc.

See section 5.4.3.6.1 of this Guidance for overlap with other EU directives.

5.4.3.5.2 Data requirements

Test methods

For efficacy testing of teat disinfection products, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a teat disinfectant:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative carrier test (phase 2, step 2), or a field trial;

all simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Phase 2, step 1 tests for the veterinary area, with relevant soiling for teat disinfection should be used.

No European standard phase 2, step 2 tests are available for teat disinfection. To demonstrate efficacy a phase 2, step 2 tests should be provided with a test design relevant for the use. The test design must reflect the application and should be discussed with and agreed by the CA before testing takes place.

When standard tests become available, which are relevant for teat disinfectants, it is recommended to use these tests.

Alternatively a phase 3 test, field trial, may be provided with a test design relevant for the use. The test design must reflect the application, should include a control with water instead of biocide, and should be discussed with and agreed by the CA before testing takes place.

<u>Disinfectant towelettes/wipes</u>
For disinfectant wipes, the phase 2, step 1 tests should be done preferably with the liquid extracted from the wipe or if difficult to extract, use the liquid as it is before it is added to the wipes. Phase 2, step 2 tests should be tests with mechanical action or, when this test is not available, with liquid extracted from the wipe (not the original liquid), with a justification of the volume that is applied per square centimetre. In addition, a test must be performed that shows that either the wipe will still disinfect after the wipe dries out or that the wipe stays wet long enough to disinfect according to the claim. In addition, the use directions can

address these issues, for instance, stating on the label that only wet wipes are efficacious, or giving expiry dates for re-sealable packages.

For an overview of available EN tests see Appendices 2 and 4.

Example of phase 2, step 2 tests
The phase 2, step 2 surface carrier test can be derived from adaptation of CEN TC 216 surface tests. Instead of a hard surface carrier, carriers involved could be made of material simulating the teat. Justification for the used carrier should be provided.

Cells of test organisms should be applied and fixed onto the surface in a manner which represents pre- and post-application, (dried in case of pre-milking or not dried in case of post-milking), and incubated with the product for the appropriate time (see EN phase 2, step 2 test, for example, EN 14349 or EN 16437, for growth conditions, controls, etc.). After incubation with the product the cell count reduction is evaluated and compared to a water control.

The test design should be discussed with and agreed by the CA before testing takes place.

Test organisms

Teat disinfection products should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is intended to be claimed.

Virucidal activity

For products used as teat disinfectants, a differentiation in the virucidal activity is made.

The claims can be:

- virucidal activity, or
- virucidal activity against enveloped viruses.

For each claim, different test organisms should be tested.

The EN 14675 test for virucidal activity in the veterinary area tests Bovine Enterovirus Type 1 (ECBO), a non-enveloped virus. When this test is passed, virucidal activity can be claimed.

Virucidal activity against enveloped viruses can be claimed when Vaccinia virus is tested in a (modified) EN 14675 test.

The SPC should clearly state which virucidal claim is demonstrated. When only virucidal activity against enveloped viruses is demonstrated the claim cannot be "virucidal activity".

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value, therefore maximum contact times are set. For post-milking teat disinfection products the contact time is normally 1 minute but should not exceed 5 minutes.

The contact time for pre-milking teat disinfection products is normally 30 seconds or less and should not exceed 60 seconds. Deviations from this contact time requirement must be justified in the application for authorisation and will be evaluated on a case-by-case basis.

Tests for pre-milking products should be carried out with either low or high-level soiling for veterinary surfaces, depending on the instructions given for pre-cleaning procedures.

Tests for post-milking products should be carried out with soiling for teat disinfectants in accordance with the test requirements. Soiling conditions for teat disinfectants are mentioned in the bactericidal test and should be used for the test with other organisms as well.

The soiling needed can be found in EN 1656 and referenced in Appendix 4.

For teat disinfection a test temperature of 30ºC or lower is acceptable. Deviations from this temperature requirement must be justified in the application and will be evaluated on a case-by-case basis.

5.4.3.5.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.6 Other animal corporal hygiene

5.4.3.6.1 Introduction

Disinfectants for animal corporal hygiene are used to disinfect the skin of animals. This section includes all animal skin disinfectants, which are not covered in the sections on teat or animal feet disinfection below.

A product applied on animal skin could be either a biocidal or a veterinary medicinal or a product for cleaning or cosmetic purposes. If the product under investigation is within the scope of the Veterinary Medicinal Products Directive (2001/82/EC as amended by 2004/28/EC) it is excluded from the BPR for the respective use. When a product does not have a biocidal claim (e.g. skin disinfection, activity against micro-organisms claimed) but only a cosmetic claim (e.g. cleaning skin, paws) it is excluded from the BPR for the respective use.

Products for disinfection of damaged skin e.g. wound disinfection, or disinfection of undamaged skin before medical treatment, e.g. pre-operative skin disinfection or disinfection before injection, are always veterinary medicinal products.

When applying for authorisation for an animal corporal hygiene biocidal product within PT3 a detailed description of the intended use should be given, to prevent authorisation of veterinary medicinal products or medicinal uses, as biocides, e.g. the claim "animal skin disinfection" is insufficient.

For products that fall under the BPR the data requirements described in the following sections apply.

5.4.3.6.2 Data requirements

Test methods

For efficacy testing of animal corporal hygiene products, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for an animal corporal hygiene disinfectant:

- a quantitative suspension test (phase 2, step 1;

- and a quantitative carrier test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Field trials in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

Phase 2, step 1 tests for the veterinary area can be used.

No European standard phase 2, step 2 tests are available for animal skin disinfection. To demonstrate efficacy a phase 2, step 2 tests should be provided with a test design relevant for the use. The test design must reflect the application and should be discussed with and agreed by the CA before testing takes place.

When standard tests become available, which are relevant for skin disinfectants, it is recommended to use these tests.

For an overview of available EN tests see Appendices 4 and 6.

Example of phase 2, step 2 tests
The phase 2, step 2 surface carrier test can be derived from adaptation of CEN TC 216 surface tests. Instead of a hard surface carrier, carriers could be made of material simulating animal skin[21]. Method are currently being developed, but their aptitude for the respective biocidal use/demonstration of efficacy for animal skin disinfectants remains to be proven. Justification for the used carrier should be provided.

Cells of test organisms could be applied to the surface, dried, and incubated with the product for the appropriate time (see EN phase 2, step 2 test, e.g. EN 14349, for growth conditions, controls, etc.). After incubation with the product the cell count reduction is evaluated and compared to a water control.

For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Animal corporal hygiene products should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value.

The claimed contact time has to be a realistic value.

It must be ensured that the test surface does not remain wet longer than the part of the animal body treated with the product, for example, by using higher (more realistic) temperatures. When residual efficacy is claimed this should be demonstrated in efficacy tests.

[21] Please take into account EU regulation 1069/2009, on animal by-products.

Tests should be carried out with high level or low level soiling conditions in accordance with the test requirements. Soiling conditions for animal corporal hygiene products are the same as for other veterinary area disinfectants. The soiling needed for clean and dirty conditions can be found in the relevant EN tests and referenced in Appendix 4.

For animal corporal hygiene products a test temperature of 30ºC or lower is acceptable. Deviations from this temperature requirement must be justified in the application and will be evaluated on a case-by-case basis.

5.4.3.6.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg_{10} reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.7 Disinfection of hatching-eggs

5.4.3.7.1 Introduction

Disinfection of hatching-eggs includes the disinfection of eggs before they hatch in hatcheries. Products are applied in a bath, as a spray, as wipes, fumigation, etc.

5.4.3.7.2 Data requirements

Test methods

For efficacy testing of disinfection products for hatching-eggs, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for a disinfectant for hatching-eggs:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative carrier test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Field trials in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

Phase 2, step 1 tests for the veterinary area can be used. As phase 2, step 2 test the EN test for porous surfaces should be used for bacteria, and the DVG guidelines for other test organisms should be used (see Appendix 4). As an alternative porous surface for disinfection of hatching-eggs, sterile eggshells may be used in phase 2, step 2 tests. If sterile eggshells are used, the recovery rate should be assessed.

For egg disinfection in a bath, the information should be provided on how long the efficacy of a bath can be guaranteed (time period, number of eggs passing through). Challenging efficacy tests, e.g. capacity tests, see section 5.4.0.4.1 of this Guidance, should be done simulating the consecutive challenge not only by micro-organisms but also by soiling. A test with relevant organic soiling should be provided in order to ensure that the biocidal product can be challenged successfully with the test organism until the end of the claimed period of use. When a challenge test is provided and the efficacy of the challenged solution has been determined at the end of the challenged period the quantitative suspension test can be waived. Alternatively, for products with one active substance that can easily be measured,

efficacy can be demonstrated using a field trial in which the amount of active substance is measured several times during the test period. Efficacy (suspension) tests should be provided with the product with the active substance concentration obtained in the field at the end of the claimed period of use.

For products applied by airborne diffusion of an aerosol, a smoke, a vapour or a gas, with the intention to disinfect the external surfaces of the eggs in the room, the test methods are described in section 5.4.3.8 Room disinfection/automated airborne disinfection of surfaces of this guidance. These tests should be adapted to fit the conditions, e.g. soiling, additional eggshell surfaces (due to the eggs and racks in the room), etc. for veterinary use. For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Disinfection products for hatching-eggs should be at least sufficiently effective against bacteria and fungal spores. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value. It must be ensured that the disinfected parts stay wet during the contact time. When residual efficacy is claimed for dried products this should be demonstrated in efficacy tests.

Tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning prior to disinfection is necessary. If this is not stated in the SPC, the test should be done under dirty conditions. Soiling conditions for hatching-eggs disinfectants are the same as for other veterinary area disinfectants. The soiling needed for clean and dirty conditions can be found in the relevant EN tests and referenced in Appendix 4.

For disinfection of hatching-eggs, a temperature of 30ºC or lower is acceptable. Deviations from this temperature requirement must be justified in the application and will be evaluated on a case-by-case basis.

5.4.3.7.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory or, when applicable, simulated-use tests or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.8 Room disinfection/automated airborne disinfection of surfaces

5.4.3.8.1 Introduction

Once animals have been removed from an enclosure, automated airborne disinfection can be used to complete surface disinfection prior to the introduction of new animals. Automated airborne disinfection can be particularly useful in the remediation of animal diseases (e.g. avian influenza, African swine fever, bovine botulism).

Room disinfection can offer an additional precaution for farms with high health status or those in which microbial pressure is very high.

Automated airborne disinfection can also take place in PT3-related areas of slaughterhouses, as expected in the hygiene plan.

The steps and key parameters of airborne disinfection are described in section 5.4.2.5.1, and test conditions and pass criteria in Appendix 4.

5.4.3.8.2 Data requirements

Test methods

Airborne disinfection differs from the direct application of liquids to surfaces. Therefore the EN phase 2, step 2 standards for surface disinfection are not applicable for room disinfection.

The following test is required for a room disinfectant:

- simulated-use test, such as EN 17272 for disinfection using the airborne application (phase 2, step 2).

The main principles of EN 17272 are described in section 5.4.2.5.2

Test organisms

Disinfection products for room disinfection should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

An overview of reference test organisms is given in Appendix 3.

5.4.3.8.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required simulated-use test has been performed (using the required test organisms and test conditions), and when the pass criteria for the test have been met.

Where pass criteria are available in the standard tests, these should be met.

If the test does not provide these criteria, the general criteria referenced in Appendix 4 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.8.4 Provisions to be taken into account

See section 5.4.2.5.4.

5.4.3.9 Textile disinfection in PT3

5.4.3.9.1 Introduction

Textile disinfection products within PT3 are mainly used to disinfect the cloths used for teat cleaning/disinfection of dairy cattle before milking. Products are normally applied by dipping the cloth in a disinfectant solution. For other uses, the requirements below should be adapted to fit the intended use.

5.4.3.9.2 Data requirements

Test methods

For efficacy testing of textile disinfection products, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for textile disinfection products:

- a quantitative suspension test (phase 2, step 1);
- a quantitative carrier test involving carriers made of test fabric (cotton, polyester) (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, repeated challenges, etc.).

Field trials in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

Test methods for textile disinfection are described in section 5.4.2.10 of this Guidance.

Currently, the following tests are available:

- phase 2, step 1 suspension tests as described in EN 14885,
- phase 2, step 2 tests involving test fabrics in:
    - a small scale laboratory setting (e.g. ASTM E2406) or;
    - a full-scale laundry machine test (EN 16616, or DGHM).

In phase 2, step 2 tests fabric is contaminated with test organisms and then exposed to the disinfectant. These tests should be adapted to fit the conditions (soiling, etc. see 4.8.2.3) for veterinary use. For disinfection in washing machines a full-scale laundry machine test, according to test conditions mentioned in section 5.4.2.10.2 of this Guidance, is obligatory.

The EN tests are strongly recommended where available and appropriate. For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Textile disinfection products should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

When the product is intended to be used at high temperatures (>40 ºC) relevant test organisms for these temperatures should be used as described in section 5.4.0.4.4 of this Guidance.

Test conditions

It is important that the tests are performed using the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value. The contact time products intended for disinfection of textile in between milking sessions can be several hours.

Tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements for the veterinary area. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning prior to disinfection is necessary. If this is not stated in the SPC, the test should be done under dirty conditions. Soiling conditions for milking-textile disinfectants are the same as for teat disinfectants. The soiling needed for

clean and dirty conditions can be found in the relevant EN standards and referenced in Appendix 4.

For textile disinfection a test temperature should be according to the use instructions. When the textile is immersed in a bucket with warm water it should be taken into account that the water temperature will decrease during the disinfection process. This should be reflected in the test conditions.

5.4.3.9.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg_{10} reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.10 Disinfection of manure, litter and other substrates for veterinary use

5.4.3.10.1 Introduction

Manure mainly consists of urines and faeces (organic matters and intestinal bacteria) in which can also be mixed straw of litters in more or less big quantity, according to the breeding technique (partial slats or complete slats).

Manure has a potential for spreading infectious diseases and biocidal products are used to destroy some infective agents and also control microbial agents responsible for malodours.

Litters are usually used in animal housing (poultry, pigsties, etc.) and also for pets in private uses. They absorb urines and faeces. Biocidal products are mainly used to deodorize and neutralize bad smells.

5.4.3.10.2 Data requirements

Test methods

For efficacy testing of disinfects biocidal products used for manure and litter disinfection, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required:

- a quantitative suspension test (phase 2, step 1),
- and simulated-use test, or field trial

all simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, mode of application, pH, etc.).

An example of a simulated-use test could be autoclaved manure or litter collected in animal housing and tested in the lab with inoculation of target organisms. A control without addition of disinfectants should be included. The test design should be discussed with and agreed by the CA before testing takes place.

In case of products claiming malodour control, the same requirements as mentioned in the section 5.4.0.5.4 of this Guidance, are required.

Test organisms

Generally, target organisms have to be representative of the veterinary area, as stated in EN 14885.

For specific uses in industry, an exception can be made when sound justification is provided. This will be evaluated on a case-by-case basis.

Taking into account the specificity of some kind of uses, it may be justified to test additional target organisms (e.g. *Brachyspira hyodysenteriae* agent of swine dysentery,), special growth conditions, etc.

In case of malodour control, tests should be performed with odour producing micro-organisms. A justification for which bacteria, fungi, etc. are relevant to the intended use should be provided. Along with these laboratory tests, an odour test can be performed.

Test conditions

It is important that the tests are performed with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value.

Quantitative suspension tests must be carried out with high level soiling conditions and a temperature of 10°C or less.

The test temperature should be according to the use instructions in the SPC and appropriate to the uses (stables, private homes, etc.).

Field and simulated-use test have to be performed according to the dose, conditions and mode of application of the product. For example, if the product is applied on top of the manure, the product does not have to be mixed with the organic matter but has to be put on top of it (to mimic the diffusion and evaluate efficacy in the same conditions as in the practice).

In case of litter, if persistence is claimed with some recommendations about the frequency of renewal, adequate simulating tests (with appropriate contribution of organic matters in the test) have to be performed.

Deviations from these requirements must be justified in the application for authorisation and will be evaluated on a case-by-case basis.

5.4.3.10.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, field (or simulated-use) tests have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT3 products the required lg_{10} reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.3.11 Other uses in PT3

Several uses of PT3 products have been specified in the above sections and data requirements and acceptance criteria for these uses are described. For products with other uses that do not fit in one of the described uses, it is up to the applicant to demonstrate efficacy in an appropriate way.

In general, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred. Where possible the standard tests required for the described uses should be taken, e.g. EN phase 2, step 1 and step 2 tests for the veterinary area. Where the tests are not appropriate for the product, other tests can be used. In that case, a justification for the relevance of the tests used should be provided. The test design should be discussed with

and agreed upon by the CA before testing takes place. The evaluation will be done on a case-by-case basis by the CAs.

5.4.4 PT4 Food and feed area disinfectants

5.4.4.1 Introduction

Product type 4 contains biocidal products used for the disinfection of equipment, containers, consumption utensils, surfaces or pipework associated with the production, transport, storage or consumption of food or feed (including drinking water) for humans and animals.

Some disinfectants applied in the food or feed area can be either biocidal product or a preservative for food or feed. If the product under investigation is within the scope of Regulations (EC) 852/2004, 853/2004 and 854/2004 on food hygiene, it is excluded from the BPR. Regulation 852/2004 is on the hygiene of foodstuffs; Regulation 853/2004 lays down specific hygiene rules for food of animal origin; Regulation 854/2004 lays down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

In the sections below the requirements and acceptance criteria for most common uses are specified. For other uses and claims that are not specifically mentioned the requirements will be set on a case-by-case basis by the CAs.

Bacteria and yeasts are mandatory target organisms for PT4. For non-professional users, it is not feasible to differentiate bacteria and yeasts as target organisms. The professional users may discriminate between bacteria and yeasts, and in the food industry, the target organisms may differ between applications and production lines. Therefore, contact time and dose can be differentiated for bacteria and yeasts for professional users, if sufficiently justified in the PAR.

5.4.4.2 Disinfection of hard surfaces in food and feed area PT4

5.4.4.2.1 Introduction

Biocides can be used to disinfect hard surfaces in areas such as food industry, kitchens in restaurants or homes, shops like butchers and grocery shops were food is processed etc. These surfaces may be tables, floors, walls, the outsides of machinery, equipment, reservoirs for water or feed in animal housing etc. Products are often wiped, sprayed, foamed, applied by low to high pressure etc., onto the surface, and maybe washed or wiped off after a certain contact time.

The testing requirements for some specific uses of hard surface disinfectants are discussed in separate sections, for example, CIP, equipment and dishwashing disinfectants etc.

5.4.4.2.2 Data requirements

Test methods

For efficacy testing of food and feed area biocidal products used on hard surfaces, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for hard surface disinfectants:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative surface test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Tests in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

Several methods for testing the efficacy of hard surface disinfectants are available. Tests with mechanical action might be adopted from the medical area, if appropriate. Appendices 2 and 4 give a list of recommended test methods.

The following documents are recommended for surface disinfection:

- EN 14885: gives an overview of which EN phase 2, step 1 and step 2 tests to use for different uses;

if CEN standards are not relevant or available for the use or organisms claimed the following documents are recommended if appropriately reflecting the application:

- OECD guidance for the testing of chemicals: Quantitative method for evaluating the activity of microbicides used on hard non-porous surfaces (these are surface tests which would be considered phase 2, step 2 tests).

The use of the specified tests is strongly recommended where they are relevant and appropriate.

When efficacy against biofilm is claimed a simulated-use test or field trial has to be provided, next to a phase 2, step 1 test. See section 5.4.2.11 of this Guidance for test methods.

Disinfectant towelettes/wipes

For disinfectant wipe, the phase 2, step 1 tests should be done preferably with the liquid extracted from the wipe, or if difficult to extract, use the liquid as it is before it is added to the wipes. Phase 2, step 2 tests should be tests with mechanical action. These tests are available for bacteria and yeasts. For testing other organisms surface tests can be done with liquid extracted from the wipe (not the original liquid), with a justification of the volume that is applied per square centimetre. In addition, a test must be performed that shows that either the wipe will still disinfect after the wipe dries out or that the wipe stays wet long enough to disinfect according to the claim. In addition, the use directions can address these issues, for instance, stating on the label that only wet wipes are efficacious, defining the surface area each towel can disinfect (e.g. 0.5 m^2), or giving expiry dates for re-sealable packages.

Test organisms

Food and feed hard surface biocidal products should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For specific uses in industry, an exception can be made when sound justification is provided. This will be evaluated on a case-by-case basis.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed. Relevant groups of test organisms, next to bacteria and yeasts, can be fungi (fungal spores), viruses, bacteriophages, and bacterial spores. Bacteriophages are mainly of importance in the dairy industry.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. For food area disinfectants *Salmonella thyphimurium*, *Salmonella* spp*., Listeria* spp*.* and *Campylobacter jejuni* are relevant target organisms. For products which claim general efficacy against bacteria, the standard test bacteria should be tested. For these products efficacy against *Salmonella* spp*., Listeria* spp*.* and *Campylobacter jejuni* is assumed, because they are more susceptible than the standard test bacteria.

The EN standards for food area only include a test on bacteriophages but not on other viruses. To demonstrate a general virus claim a modified EN phase 2, step 1 test (medical area test with food area soiling) can be provided with Adenovirus and Murine Norovirus as test organism and a phase 2, step 2 test (either modified EN medical test, or DVG test or, as soon as available, an EN food area test) with Murine Norovirus.

An overview of reference test organisms is given in Appendix 3.

Test conditions

It is important that the tests are performed with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value.

Tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning prior to disinfection is necessary. If this is not stated in the SPC the test should be done under dirty conditions. Note that for use in specific industries different types of soiling for dirty conditions should be used.

The soiling needed for clean and dirty conditions can be found in the relevant EN tests or EN 14885 (version 2014 or later) and referenced in Appendix 4.

If a product is intended to be used in more than one area of use (e.g. milk industry and meat industry) it is justified, after having identified the most challenging test organism, to test the relevant soiling types with this organism. That applies only per group of organisms (e.g. bacteria).

The test temperature should be according to the use instructions in the SPC. Food and feed area disinfectants are generally used at room temperature (test temperature 20ºC) but for some uses and claims (e.g. surfaces in cold storage rooms) low temperatures of 4ºC or 10ºC are relevant and should be tested.

5.4.4.2.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT4 products the required lg_{10} reductions are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.4.3 Room disinfection/automated airborne disinfection of surfaces

5.4.4.3.1 Introduction

The food industry is very concerned about the problems related to the cleaning and disinfection of premises to fight against different sources of contamination. These operations aim to eliminate dirt as well as contamination and infections of microbiological and chemical origin.

Automated room disinfection is an integral part of hygiene plans in these industries.

The steps and key parameters of airborne disinfection are described in section 5.4.2.5.1

5.4.4.3.2 Data requirements

Test methods

Airborne disinfection differs from the direct application of liquids to surfaces. Therefore the EN phase 2, step 2 standards for surface disinfection are not applicable for room disinfection.

The following test is required for a room disinfectant:

- simulated-use test, such as EN 17272 for disinfection using the airborne application (phase 2, step 2).

The main principles of EN 17272 are described in section 5.4.2.5.2

Test organisms

Disinfection products for room disinfection should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For specific uses in industry, an exception can be made when sound justification is provided. This will be evaluated on a case-by-case basis.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed. Relevant groups of test organisms, next to bacteria and yeasts, can be fungi (fungal spores), viruses, bacteriophages, and bacterial spores. Bacteriophages are mainly of importance in the dairy industry.

An overview of reference test organisms is given in Appendix 3.

5.4.4.3.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required simulated-use test has been performed (using the required test organisms and test conditions), and when the pass criteria for the test have been met.

Where pass criteria are available in the standard tests, these should be met.

If the test does not provide these criteria, the general criteria referenced in Appendix 4 can be taken as guidance for the level of reduction required. Deviations from the pass criteria are possible but must be justified in the application.

5.4.4.3.4 Provisions to be taken into account

See section 5.4.2.5.5.

5.4.4.4 Disinfection of inner surfaces in PT4

5.4.4.4.1 Introduction

Biocides can be used to disinfect the inner surfaces of pipes, tanks, fillers, mixers, and other machines which come in contact with food or feed (including liquids). This includes food and feed industry, milking equipment on farms, large equipment in restaurants or shops where food is processed, etc. Inner surfaces in contact with water are discussed in the following sections.

These surfaces are disinfected by filling and circulating the biocide in the pipes, tanks, machines, etc. with disinfectant (Cleaning In Place, CIP). Also disinfection of inner surfaces of equipment by filling without circulation (not using CIP) is included in this section.

5.4.4.4.2 Data requirements

Test methods

For efficacy testing of food and feed area biocidal products used on inner surfaces using CIP, the following tests are required:

- quantitative suspension tests (phase 2, step 1), simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

For efficacy testing of food and feed area biocidal products used on inner surfaces by filling without circulation, the following tests are required for these disinfectants:

- quantitative suspension tests (phase 2, step 1);
- and a quantitative surface test (phase 2, step 2);

both simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Tests in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

Several methods for testing the efficacy of inner surface disinfectants are available. Appendices 2 and 4 give a list of recommended test methods.

The following documents are recommended for inner surface disinfection using CIP:

- EN 14885 gives an overview of which EN phase 2, step 1 and step 2 tests to use for different uses;

if CEN standards are not relevant or available for the use or organisms claimed the following documents are recommended if appropriately reflecting the application:

- OECD guidance for the testing of chemicals: Quantitative method for evaluating the activity of microbicides used on hard non-porous surfaces. (These are surface tests which would be considered phase 2, step 2 tests).

The use of the specified tests is strongly recommended where they are relevant and appropriate.

When efficacy against biofilm is claimed a simulated-use test or field trial has to be provided, next to a phase 2, step 1 test. See section 5.4.2.11 of this Guidance for test methods.

When the disinfection is done with vaporised biocide a simulated-use test or a field trial has to be provided. See section 5.4.2.5 of this Guidance for test methods.

Test organisms

Food and feed hard surface biocidal products should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For specific uses in industry, an exception can be made when sound justification is provided. This will be evaluated on a case-by-case basis.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed. Relevant groups of organisms, next to bacteria and yeasts, can be fungal spores, viruses, phages, and bacterial spores. Phages are mainly of importance in the dairy industry.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. For food area disinfectants *Salmonella* spp*., Listeria* spp*.* and *Campylobacter jejuni* are relevant target organisms. For products that claim general efficacy against bacteria, the standard test bacteria should be tested. For these products efficacy against *Salmonella* spp*.,*

Listeria spp. and *Campylobacter jejuni* is assumed, because they are more susceptible than the standard test bacteria.

The EN standards for food area only include a test on bacteriophages but not on other viruses. To demonstrate virucidal activity a modified EN phase 2, step 1 and phase 2, step 2 test (medical area test with food area soiling) can be provided with Adenovirus and Murine Norovirus (phase 2, step 1 test) and with Murine Norovirus (phase 2, step 2 test) as test organisms.

When CIP is done at high temperatures relevant test organisms for these temperatures should be used as described in section 5.4.0.4.4 of this Guidance.

An overview of test organisms, also for high temperatures, is given in Appendix 3.

Test conditions

It is important that the tests are performed with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value.

Tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning prior to disinfection is necessary. If this is not stated in the SPC the test should be done under dirty conditions. Note that for use in specific industries different types of soiling for dirty conditions should be used.

The soiling needed for clean and dirty conditions can be found in the relevant EN tests or EN 14885 (version 2014 or later) and referenced in Appendix 4.

The test temperature should be according to the use instructions in the SPC. Food and feed area disinfectants are generally used at room temperature (test temperature 20 ºC) but for some uses and claims other temperatures are relevant. For example, for surfaces in cold machinery, low temperatures of 4 ºC or 10 ºC are relevant and should be tested. CIP disinfection is often done at high temperatures of 40 to 80 ºC. When this is the intended use the test temperature should be in accordance with the use and relevant test organisms should be used (see section 5.4.4.3.2 of this Guidance).

5.4.4.4.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, when applicable, simulated-use or field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard tests, these should be met. For PT4 products the required lg reduction tests are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.4.5 Equipment disinfection by soaking

5.4.4.5.1 Introduction

Biocides can be used to disinfect dishes, equipment, crates, boxes, etc. by soaking. This can include dishwashing disinfectants, however, normal dishwashing detergents are cleaning products and not included in the BPR. Equipment disinfection in washing machines is covered in the next section.

This can be used in areas such as food industry, kitchens in restaurants or homes, shops like butchers and grocery shops were food or feed is processed, etc.

5.4.4.5.2 Data requirements

Test methods

For efficacy testing of equipment and dishwashing disinfectants, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for these disinfectants:

- a quantitative suspension test (phase 2, step 1;
- and a quantitative surface test (phase 2, step 2);

both tests simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Tests in phase 3 are optional, according to section 5.4.0.4.3 of this Guidance. No validated test methods are available yet.

For equipment disinfection by soaking, the information should be provided on how long the efficacy of a solution can be guaranteed. Challenging efficacy tests, e.g. capacity tests, see section 5.4.0.4.1 of this guidance, should be done simulating the consecutive challenge not only by micro-organisms but also by soiling.

Several methods for testing the efficacy of hard surface disinfectants are available.

Appendices 2 and 4 give a list of recommended test methods.

The following documents are recommended for equipment and dishwashing disinfection:

- EN 14885: gives an overview of which EN phase 2, step 1 and step 2 tests to use for different uses,

if CEN standards are not relevant or not available for the use or organisms claimed the following documents are recommended if appropriately reflecting the application:

- OECD guidance for the testing of chemicals: Quantitative method for evaluating the activity of microbicides used on hard non-porous surfaces. (These are surface tests which would be considered phase 2, step 2 tests)

The use of the specified tests is strongly recommended where they are relevant and appropriate.

When efficacy against biofilm is claimed a simulated-use test or a field trial has to be provided, next to a phase 2, step 1 test. See section 5.4.2.11 of this Guidance for test methods.

Test organisms

Equipment and dishwashing disinfectants should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For specific purposes in industrial uses, an exception can be made when sound justification is provided. This will be evaluated on a case-by-case basis.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed. Relevant groups of test organisms, next to bacteria and yeasts, can be fungi (fungal spores), viruses, bacteriophages, and bacterial spores. Bacteriophages are mainly of importance in the dairy industry.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. For dish-washing disinfectants *Salmonella* spp*., Listeria* spp*.* and *Campylobacter jejuni* are relevant target organisms. For products which claim general efficacy against

bacteria, the standard test bacteria should be tested. For these products efficacy against *Salmonella* spp*., Listeria* spp*.* and *Campylobacter jejuni* is assumed because they are more susceptible than the standard test bacteria.

The EN standards for food area only include a test on bacteriophages but not on other viruses. To demonstrate virucidal activity a modified EN phase 2, step 1 and phase 2, step 2 test (medical area test with food area soiling) can be provided with Adenovirus and Murine Norovirus (phase 2, step 1 test) and with Murine Norovirus (phase 2, step 2 test) as test organisms.

When the product is intended to be used at high temperatures (>40 ºC) relevant test organisms for these temperatures should be used as described in section 5.4.0.4.4 of this Guidance.

An overview of reference test organisms, also for high temperatures, is given in Appendix 3.

Test conditions

It is important that the tests are performed with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value. For manual dishwashing disinfectants, the contact time will be short (seconds), while industrial equipment disinfection by soaking in a solution can be very long (hours).

In general dishwashing disinfectants should be tested under dirty conditions since these products are mainly used for combined cleaning and disinfection. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning prior to disinfection is necessary. If this is not stated in the SPC the test should be done under dirty conditions.

Tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements.

Note that for use in specific industries different types of soiling for dirty conditions should be used. The soiling needed for clean and dirty conditions can be found in the relevant EN tests or EN 14885 (version 2014 or later) and referenced in Appendix 4.

The test temperature should be according to the use instructions in the SPC.

Dishwashing disinfectants for manual use are normally used at 40ºC and therefore tests should be done at this temperature. When the product is used at lower temperatures (e.g. only for rinsing after normal dishwashing with hot water) tests can be done at 20ºC. When the intended use is soaking, starting with hot water and after which the solution will cool down during the contact time, this should also be taken into account in the tests.

When disinfection is done at temperatures of 40 to 80 ºC the test temperature should be in accordance with the use and relevant test organisms should be used (see section 5.4.4.4.2 of this Guidance).

5.4.4.5.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and, when applicable, simulated-use tests or field trial have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT4 products the required lg_{10} reductions tests are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.4.6 Disinfection in dish washing machines and crate washers

5.4.4.6.1 Introduction

Biocides can be used to disinfect dishes, equipment, crates, boxes, etc. in industrial or dishwashing machines.

This can be used in areas such as food or feed industry, kitchens in restaurants or homes, shops like butchers and grocery shops were food is processed, etc.

5.4.4.6.2 Data requirements

Test methods

For efficacy testing of equipment and dish washing disinfectants the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following tests are required for these disinfectants:

- a quantitative suspension test (phase 2, step 1);
- and a quantitative surface test (phase 2, step 2);
- and a simulated-use test or a field trial (phase 3) for disinfectants used in (dish)washing machines;

all tests simulating practical conditions appropriate to its intended use (temperature, soiling, different surfaces, contact time, etc.).

Several methods for testing the efficacy of hard surface disinfectants are available.

Appendices 2 and 4 give a list of recommended test methods.

The following documents are recommended for surface disinfection in dish washing machines:

- EN 14885: gives an overview of which EN phase 2, step 1 and step 2 tests to use for different uses,

The following test might be helpful for designing simulated-use or field trials:

- DIN SPEC 10534.

Test organisms

Equipment and dishwashing disinfectants should be at least sufficiently effective against bacteria and yeasts. Efficacy tests with these organisms should always be provided.

For uses in industrial dishwashers for specific purposes, an exception can be made when sound justification is provided. This will be evaluated on a case-by-case basis.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed. Relevant groups of test organisms, next to bacteria and yeasts, can be fungi (fungal spores), viruses, bacteriophages, and bacterial spores. Bacteriophages are mainly of importance in the dairy industry.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. For dishwashing disinfectants *Salmonella* spp*., Listeria* spp*.* and *Campylobacter jejuni* are relevant target organisms. For products which claim general efficacy against bacteria, the standard test bacteria should be tested. For these products efficacy against *Salmonella* spp*., Listeria* spp*.* and *Campylobacter jejuni* is assumed because they are more susceptible than the standard test bacteria.

The EN standards for food area only include a test on bacteriophages but not on other viruses. To demonstrate virucidal activity a modified EN phase 2, step 1 and phase 2, step 2 test (medical area test with food area soiling) can be provided with Adenovirus and Murine Norovirus (phase 2, step 1 test) and with Murine Norovirus (phase 2, step 2 test) as test organisms.

When the product is intended to be used at high temperatures (>40 ºC) relevant test organisms for these temperatures should be used as described in section 5.4.0.4.4 of this Guidance.

An overview of reference test organisms, also for high temperatures, is given in Appendix 3.

Test conditions

It is important that the tests are performed with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value. It will depend on the contact time for the disinfection cycle in (dish)washing machines. Justification for the used contact time should be given.

In general, dishwashing disinfectants should be tested under dirty conditions since these products are mainly used for combined cleaning and disinfection. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning prior to disinfection is necessary or when this is incorporated in a previous cycle of the (dish)washing machine. If this is not stated in the SPC the test should be done under dirty conditions.

Tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements.

Note that for use in specific industries different types of soiling for dirty conditions should be used.

The soiling needed for clean and dirty conditions can be found in the relevant EN tests or EN 14885 (version 2014 or later) and referenced in Appendix 4.

For products intended to be added to (dish)washing machines, information on the following in-use conditions should be provided:

- the concentration of the product (or at least the active substance) in the water during disinfecting process (i.e. washing or rinsing). The water volume used can differ between wash and rinse cycle and different washing programmes, but also between dish washing machines;
- the water to dishes ratio in the test is an important factor that should reflect the in-use conditions;
- the temperature during the disinfection process (high when added in wash process, low in rinse process);
- the contact time (differs between various washing programmes and washing machines).

The laboratory tests should be performed under these conditions. The conditions for effective disinfection can normally only be carried out in professional dish washing machines.

If the exact conditions cannot be met, for example, in household machines, reasonable worst case conditions must be tested.

Worst case conditions, e.g.:

- the lowest temperature;

- the highest volume of water (i.e. maximum dilution of the product);
- the shortest contact time;
- the maximum load of dishes (i.e. smallest water to dishes ratio).

The test temperature should be according to the use instructions in the SPC.

When the product is used at lower temperatures (e.g. only for rinsing after normal dish washing with hot water) tests can be done at 20ºC. When disinfection is done at temperatures of 40 to 80 ºC the test temperature should be in accordance with the use and relevant test organisms should be used (see section 5.4.4.5.2. of this Guidance).

5.4.4.6.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests or field trial have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT4 products the required lg_{10} reductions tests are referenced in Appendix 4.

Deviations from the pass criteria are possible but must be justified in the application.

5.4.4.7 Disinfection of inner surfaces in human drinking water systems

5.4.4.7.1 Introduction

Biocides can be used to disinfect surfaces in human drinking water systems (further referred to as drinking water). This can be large water systems in drinking water companies, transport pipes in between drinking water companies (semi-finished product), the communal piping system, collective drinking water systems (hospitals and other healthcare facilities, hotels, penitentiary institutions, etc.), and tanks and reservoirs for drinking water (for instance on ships).

When water systems are disinfected in closed circuits, after which the system is washed with clean water, it is considered to be disinfection of the pipework and is included in PT4. When disinfection is performed in water systems while they are in service and the water is also disinfected, the application is considered to be included in PT5.

The drinking water systems may be new or rehabilitated drinking water pipes (e.g. in newly built or renovated houses) or systems that are in service for some time and have become contaminated during this period.

The main need to clean and disinfect the systems is to get a fresh start of the system. Cleaning and disinfection programmes may be combined to treat these systems.

The systems that have been in service for some time may contain biofilms and organisms to be controlled might be hidden in them. For instance, *Legionella* can multiply in the biofilm.

5.4.4.7.2 Data requirements

Test methods

For efficacy testing of biocidal products used on inner surfaces of drinking water systems, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

For combined cleaning and disinfecting of drinking water pipes, the following tests are required:

- a quantitative suspension test (phase 2, step 1).
- a quantitative surface test (phase 2, step 2).

When efficacy against *Legionella* is claimed, the following tests are required:

- a quantitative suspension test (phase 2, step 1);
- a simulated-use test (phase 2, step 2) or a field trial (phase 3).

all simulating practical conditions appropriate to its intended use (temperature, soiling, contact time, etc.).

When efficacy against biofilms is claimed, the following tests are required:

- a quantitative suspension test (phase 2, step 1);
- a simulated-use test or a field trial.

Laboratory tests

EN phase 2, step 1 tests for the food industrial, domestic and institutional area are relevant for this use. Efficacy against *Legionella* can be tested in EN 13623 (phase 2, step 1).

See section 5.4.2.11 of this Guidance for biofilm test methods.

Appendices 2 and 4 give a list of recommended test methods.

Simulated-use tests or field trials

For products which claim efficacy against *Legionella,* a simulated-use test or field trial should be submitted. For a field trial the following requirements should be provided:

- before testing it should be established that the installation contains high numbers of *Legionella* (>100 cfu/L). A zero-time measurement should be performed. Systems must not be inoculated with micro-organisms in order to perform the efficacy test;
- a field trial should be performed in a system that has been in service for some time and has become infected during this period;
- the number of sampling points per location will depend on the number of draw-off points in the installation. The table below should be used.

Table 12: Number of sampling points

Number of draw-off points (outlets)	Number of sampling points
10-100	4
101 – 200	6
201 – 400	8
401 – 800	10
801 – 1600	12
> 1600	14

* a draw-off point is a point where drinking water, household water or warm water is made available for use.

- after disinfection and subsequent washing of the system with clean water (removal of disinfectant), samples should be taken and the amount of bacteria (general) and *Legionella* in the water should be determined. Samples should be taken 48 hours and 2 weeks after disinfection;
- after treatment, water from none of the sampling points should contain more than 100 colony forming units/litre *Legionella*.

Test organisms

Biocidal products for drinking water disinfection should be at least sufficiently effective against bacteria. The test organisms used in efficacy tests are stated in the applicable standard test methods. Efficacy tests with these organisms should always be provided.

For products which claim efficacy against *Legionella,* a test with *Legionella* spp. should also be performed.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

Test conditions

It is important that the tests are performed with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value.

Laboratory phase 2, step 1 tests should be carried out with soiling for clean conditions in accordance with the test requirements. The soiling needed for clean conditions can be found in the relevant EN tests and referenced in Appendix 4. Simulated-use tests should be performed with relevant soiling.

5.4.4.7.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory, or when applicable, field trials have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For these products the required lg_{10} reductions in the laboratory tests are referenced in Appendix 4. The field trial should not contain more than 100 colony forming units *Legionella* per litre.

5.4.4.8 Disinfection of inner surfaces in veterinary water systems

5.4.4.8.1 Introduction

Biocides can be used to disinfect surfaces in veterinary water systems in farms, bio-industry, etc. These are water systems that provide water for animals to drink, to prepare feed, and to use for cleaning the area. Water systems that are also suitable for human drinking water are not included in this section (see the previous section of this Guidance).

When water systems are disinfected in closed circuits, after which the system is washed with clean water, it is considered to be disinfection of the pipework and is included in PT4. When disinfection is performed in water systems while they are in service and the water is also disinfected, the application is considered to be included in PT5.

The water of these systems can be provided by drinking water companies but can also contain well, ground, or ditch water that is pumped up at the location, or other water. Water systems in livestock farming can be used to supply food additives or antibiotics to the animals. Therefore, these veterinary water systems may be more fouled than human drinking water systems.

5.4.4.8.2 Data requirements

Test methods

For the combined cleaning and disinfecting of veterinary drinking water pipes (e.g. water tanks, water in animal housings etc. used as drinking water for animals and for other uses in stables like cleaning, preparing feed, etc.), efficacy should be demonstrated in a tiered

approach as described in section 5.4.0.4.1 of this Guidance. This includes a phase 2, step 1 and step 2 test.

The following documents are recommended for disinfecting of veterinary drinking water pipes:

- EN 14885 gives an overview of which EN phase 2, step 1 and step 2 tests to use for different uses, the tests (bactericidal) for the food area are relevant for this use;

if CEN standards are not relevant or available for the use or organisms claimed the following documents are recommended if appropriately reflecting the application:

- OECD guidance for the testing of chemicals: Quantitative method for evaluating the activity of microbicides used on hard non-porous surfaces. (These are surface tests which would be considered phase 2, step 2 tests).

The use of the specified tests is strongly recommended where they are relevant and appropriate.

When efficacy against biofilms is claimed, a simulated-use test or field trial has to be performed, as well as a phase 2, step 1 test. See section 5.4.2.11 of this Guidance for test methods.

Test organisms

Biocidal products for drinking water disinfection should be at least sufficiently effective against bacteria. The test organisms used in efficacy tests are stated in the applicable standard test methods. Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only have to be provided when activity against those organisms is claimed.

Test conditions

It is important that the tests are performed with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value.

Laboratory tests should be carried out with soiling for clean or dirty conditions in accordance with the test requirements for the food area. Tests under clean conditions will only suffice when the instructions in the SPC state that cleaning of the water systems prior to disinfection is necessary. If this is not stated in the SPC the test should be done under dirty conditions.

5.4.4.8.3 Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory, or when applicable, simulated-use tests or field trial have been performed (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For these products, the required lg reductions in the laboratory tests are referenced in Appendix 4.

5.4.4.9 Other uses in PT4

Several uses of PT4 products have been specified in the above sections and data requirements and acceptance criteria for these uses are described. For products with other uses, that do not fit in one of the described uses, it is up to the applicant to demonstrate efficacy in an appropriate way.

In general, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred. Where possible the standard tests required for the described uses should be taken (e.g. EN phase 2, step 1 and step 2 tests for food area). Where the tests are not appropriate for the product, other tests can be used. In that case, a justification for the relevance of the tests used should be provided. The test design should be discussed with and agreed upon by the CA before testing takes place. The evaluation will be done on a case-by-case basis by the CAs.

5.4.4.9.1 Disinfection of packaging before aseptic filling

Several uses of PT4 products have been specified in the above sections and data requirements and acceptance criteria for these uses are described. For products with other uses, that do not fit in one of the described uses, it is up to the applicant to demonstrate efficacy in an appropriate way.

In general, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred. Where possible the standard tests required for the described uses should be taken (e.g. EN phase 2, step 1 and step 2 tests for food area). Where the tests are not appropriate for the product, other tests can be used. In that case, a justification for the relevance of the tests used should be provided. The test design should be discussed with and agreed upon by the CA before testing takes place. The evaluation will be done on a case-by-case basis by the CAs.

Products are used for the disinfection of a big variety of food/beverage/containers, e.g. PET bottles prior to filling with food/beverage, etc. The disinfection of the packaging takes place inside filling machines and typically applies at higher temperature (≥ 60°C) and in a very short contact time (from less than 1 to several seconds). Disinfectants are applied via spraying, fogging or bathing and are removed before filling the packaging.

The following data should be provided to demonstrate efficacy of a product for aseptic packaging applications:

1. Efficacy should be demonstrated by validation of the product in the disinfection process using aseptic filling devices and packaging material that are representative for the intended use of the product. Phase 2, step 1 and phase 2, step 2 tests are not required;
2. A negative control with all claimed target organisms should be performed (with e.g. water) to demonstrate that the high temperature alone is insufficient to achieve sufficient control of microorganisms. Since it might be expected that bacterial spores survive the use conditions, it can be possible to exclude a negative control for bacterial spores if sufficient scientific justification is provided;
3. Products are efficacious under certain conditions, e.g. temperature, concentration, contact time, etc. Products can be tested in aseptic filling machines that meet/use the (worst-case) conditions for the product to be efficacious. The conditions to be taken into account and reflected in the test report:
    - surface temperature;
    - concentration;
    - amount of product applied;
    - contact time;
    - relative humidity;
    - dose/application rate;

- inner surface properties of the packaging

Generally, only bacterial spores survive these conditions, while vegetative bacteria and yeasts will be killed in the negative control. Therefore, demonstrating efficacy against bacterial spores (e.g. *Geobacillus stearothermophilus*) is sufficient for an efficacy claim against other groups of microorganisms for disinfection of packaging before filling. However, when the negative control shows the survival of any other target organisms (e.g. fungal spores) these should also be tested by validation of the product in the disinfection process.

Test protocols for hygienic/aseptic devices according to class III, IV and V have been published by the Association of German Machinery and Plant Constructions (VDMA). Appropriate test protocols to demonstrate efficacy can be developed based on these VDMA methods. The use "Disinfection of packaging before filling" can be described using the combined description of class III, IV and V machines from VDMA guideline Nr. 2 (see below). The microbiological challenge test (VDMA guideline Nr. 12) is acceptable as the minimum efficacy requirement. More detailed information than that described in VDMA guideline Nr. 6 should be included in the test report, e.g., a dose of disinfectant, relative humidity, contact time, temperature, information on the cleaning of the materials prior to the disinfection procedure and surface properties of packaging material.

VDMA guidelines:

- Hygienic Filling Machines for Liquid and Viscous Foods - Classification and Typical Fields of Application. VDMA Nr. 2, 3rd revised edition 2016. https://nuv.vdma.org/documents/256988/27627144/FS_2_2000_revision%25202016_English_1544605336254.pdf/610cd0cf-57b3-fe35-c597-9f7a235f827c
- Guideline to Checking the Microbiological Safety of Hygienic Filling Machines of VDMA Classes IV and V. VDMA Nr. 12. / October 2007, 2nd revised edition 2020 https://www.vdma.org/documents/34570/15113577/FS_12_2007_english_revision+2020.pdf/bf92403f-140a-6445-c68a-c6eadf25a177?t=1618997472915
- Code of Practice Filling Machines of VDMA Hygienic Class V: Testing the Effectiveness Packaging Sterilization Devices. VDMA Nr. 6, English edition September 2008. https://www.vdma.org/documents/34570/15113577/FS_06_2002_English_revision+2008.pdf/ec84b446-0646-5110-27a2-4da4d7c14295?t=1618997471444
- External Sterilization of Packaging Materials VDMA Nr. 14, English edition July 2007. https://www.vdma.org/documents/34570/15113577/FS_14_%C3%BCberarbeitung+2020_englisch.pdf/5e1a7a1d-b384-c07d-dbcc-79bb3fdc0a2c?t=1618997473348
- Code of Practice Testing Aseptic Plants: Sterilizing the Sterile Zone in a Machine Interior VDMA Nr. 8. / 2003, 2nd revised edition July 2014 (English edition) https://www.vdma.org/documents/34570/15113577/FS_08_2003_%C3%BCberarbeitung+2014_englisch.pdf/ab4ea894-c933-2f8e-5301-59bd172b4d74?t=1618997471891
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5.4.5 PT5 Drinking water disinfectants

5.4.5.1 Introduction

Product Type 5 contains biocidal products used for the disinfection of drinking water for both humans and animals. The definition of drinking water is in accordance with Article 2 of Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. In this Guidance (and section) the term drinking water for humans is not only used for water that will be consumed directly by humans but also for other uses of water coming out of the plumbing system like showering, cooking, etc.

When disinfection is done in the water system while it is in service and the water itself is also disinfected, this is included in PT5. When water systems are disinfected in closed circuits, after which the system is washed with clean water, this is disinfection of the pipework only and is as such included in PT4.

Disinfectant products can be added to drinking water, intermittently by shock dosing or continually dosing. The purpose of this type of disinfection is to disinfect the water in order to prevent the transmission of water-borne diseases via drinking water. Water-borne transmitted pathogens can be bacteria, viruses, yeasts, fungal spores or protozoan parasites. Disinfection is only one aspect of drinking water treatment. The application of drinking water disinfectants is accompanied by the responsibility to also control any toxic disinfectant by-products. Treatment substances should only be added for specific hygienic or technical reasons, limiting application to the minimum volumes that are absolutely necessary for achieving the targeted effect (principle of minimisation) and only under conditions optimising their efficacy.

Disinfection within PT5 can be divided into six application groups:

1. Disinfection at the drinking water suppliers and their water distribution systems
2. Disinfection of raw water for individual supply (1-2 premises)
3. Disinfection in collective drinking water systems
4. Disinfection of water in reservoirs
5. Disinfection of water of undefined quality for small-scale use (up to 5 L/person/day)
6. Disinfection of water for animals

In the sections below a detailed description of each group as well as the requirements and acceptance criteria for most common uses are specified. For other uses and claims that are not specifically mentioned the requirements will be set on a case-by-case basis by the CAs.

5.4.5.2 Disinfection at the drinking water suppliers and their water distribution systems

5.4.5.2.1 Introduction

This is the disinfection of water during drinking water treatment in water plants of drinking water suppliers, during transport in between drinking water suppliers, and prior to distribution into (part of) the communal piping system (referred to as primary disinfection in this guidance). This group also includes products that are added by drinking water suppliers to the previously-treated water already in the public distribution network to ensure that an adequate disinfectant residual is maintained throughout the system (referred to as secondary disinfection in this guidance).

Following physical treatment of water, primary disinfection describes the main disinfection method employed to inactivate waterborne pathogenic micro-organisms. Primary disinfection is often supplemented by downstream secondary disinfection to maintain a residual level of disinfectant within the distribution system in order to assure good quality of drinking water to the point of compliance i.e. the consumer's tap as determined in the Drinking Water Directive (DWD).

5.4.5.2.2 Data requirements

Test methods

For product authorisation of drinking water disinfectants used by the drinking water suppliers and in water distribution systems, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

Next to a suspension test, a simulated-use test should be performed. For suspension tests EN phase 2, step 1 tests are preferred. Since for most target organisms there are no specific EN tests for drinking water disinfection, tests should be modified to reflect the use conditions with respect to soiling, temperature range and contact time. EN tests from food and industrial area (see EN 14885) can be modified (see 'Test conditions'). For virucidal activity EN 14476 can be modified.

For the simulated-use test for primary disinfection, a detailed appropriate test method is given in the test method "Quantitative determination of the efficacy of drinking water disinfectants" (see Appendix 2 Table 41). The test is performed on an adapted test rig. A disinfectant neutralizer or filter system is required to stop a reaction between disinfectant and test organisms. Currently, the simulated-use test can only be performed in the test lab in Germany where the test was developed, as only there the required test set up is available. Alternative methods will be considered and are acceptable provided they are scientifically justified and will be evaluated by the CA on a case-by-case basis. Please note that monitoring data can only be accepted as supplementary data since this data does not offer the possibility to calculate the lg reduction to evaluate the disinfection.

For secondary disinfection a simulated-use test is required with relevant use conditions with respect to temperature, soiling and contact time.

For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Drinking water disinfectants used on-site at the drinking water suppliers and water distribution systems should be at least sufficiently effective against bacteria and viruses.

Efficacy tests with these organisms should always be provided. For all other groups of organisms (Protozoa, etc.), data only have to be provided when activity against those organisms is claimed. The test organisms used in efficacy tests are normally stated in the applicable standard test methods or the test method "Quantitative determination of the efficacy of drinking water disinfectants". For drinking water disinfectants used on-site at drinking water suppliers and water distribution systems *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus hirae* and *Escherichia coli* should be tested.

In an EN suspension test, the efficacy against enteroviruses and Norovirus should be tested. In the simulated-use test bacteriophages are used as an indicator for human viruses as given in the test method "Quantitative determination of the efficacy of drinking water disinfectants".

An overview of reference test organisms is given in Appendix 3.

Test conditions

It is important that the efficacy tests are carried out with the contact time as claimed in the SPC, and also that the claimed contact time is a realistic value.

Suspension tests may be modified considering the type of disinfectant application and considering especially that the test needs to be performed reflecting the worst-case conditions (temperature, soiling, contact time, mineralization, pH). Further details can be taken from Appendix 4. For suspension tests, the maximum contact time is 30 minutes. For simulated-use tests contact times of 10 and 25 minutes should be applied.

Laboratory tests should be carried out with appropriate soiling. For primary disinfection, it can be expected that soiled water is used e.g. surface water. Therefore, for this use, the laboratory tests should be done under dirty conditions. Secondary disinfection is done on clean water, simulated by clean test conditions. Appendix 4 states the appropriate soiling for PT5.

The applicant should provide the rationale for the choices made.

Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT5 products the required lg reductions in suspension tests are referenced in Appendix 4.

The pass criteria for the simulated-use test are stated in the test (see Appendix 4). The same criteria are valid for both primary and secondary disinfection.

Deviations from the pass criteria are possible but must be justified in the application. If the simulated-use test passed but the suspension test did not pass, the applicant needs to justify why the concentration used in the simulated-use test should be considered as the effective dose.

Based on the current information there is enough evidence that the active chlorine-based products (most widely used water disinfectants), cannot pass these phase 2, step 1 tests against bacteria and viruses at typical use concentrations that have long been established. In addition, the active chlorine concentration in drinking water cannot be increased to a level that passes these criteria. Consequently, the modified phase 2, step 1 tests are considered as not obligatory for PT 5 active chlorine-based disinfectants. Efficacy of such products should be demonstrated with a simulated-use test and/or a field trial.

5.4.5.3 Disinfection of raw water for individual supply (1-2 premises)

5.4.5.3.1 Introduction

These are disinfectants intended to be used for private water supply, (i.e. any water supply which is supplied to a property that is not provided by a water supplier). Most of these supplies are situated in remote, rural parts of a country and can originate from a range of sources including wells, natural springs and watercourses.

5.4.5.3.2 Data requirements

Test methods

For product authorisation of drinking water of individual supply, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

Next to a suspension test a simulated-use test should be performed. For suspension tests EN phase 2, step 1 tests are preferred. Since for most target organisms there are no specific EN tests for drinking water disinfection, tests should be modified to reflect the use conditions with respect to temperature range, soiling and contact time. EN tests from food and industrial area (see EN 14885) can be modified (see 'Test conditions'). For virucidal activity EN 14476 can be modified.

For the simulated-use test, a detailed appropriate test method is given in the test method "Quantitative determination of the efficacy of drinking water disinfectants". The test is performed on an adapted test rig. A disinfectant neutralizer or filter system to stop a reaction between disinfectant and test organisms is required. Currently, the simulated-use test can only be performed in Germany. Alternative methods will be considered and are acceptable provided they are scientifically justified and will be evaluated by the CA on a case-by-case basis.

For an overview of available EN tests see Appendices 2 and 4.

Test organisms

Drinking water disinfectants of raw water for individual supply should be at least sufficiently effective against bacteria and viruses. Efficacy tests with these organisms should always be provided. For all other groups of organisms (Protozoa, etc.), data only have to be provided when activity against those organisms is claimed.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods or the test method "Quantitative determination of the efficacy of drinking water disinfectants". For drinking water disinfectants used in private drinking water supply systems *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus hirae* and *Escherichia coli* should be tested.

In EN suspension tests efficacy against enteroviruses and Norovirus should be tested. In the simulated-use test, bacteriophages are used as an indicator for human viruses as given in the test method "Quantitative determination of the efficacy of drinking water disinfectants".

An overview of reference test organisms is given in Appendix 3.

Test conditions

It is important that the efficacy tests are carried out with the contact time as claimed in the SPC, and also that the claimed contact time is a realistic value.

Suspension tests may be modified considering the type of disinfectant application and considering especially that the test needs to be performed reflecting the worst-case conditions (temperature, soiling, contact time, mineralization, pH). Further details can be taken from Appendix 4. For suspension tests, the maximum contact time is 30 minutes. For simulated-use tests contact times of 10 and 25 minutes should be applied.

Laboratory tests should be carried out with soiling for dirty conditions as defined in Appendix 4. Depending on the water source interfering substances may be variable and require modifications of the soiling in the efficacy tests. The applicant should provide the rationale for the choices made.

Further details can be taken from Appendix 4.

Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT5 products the required lg reductions in suspension tests are referenced in Appendix 4.

The pass criteria for the simulated-use test are stated in the test.

Deviations from the pass criteria are possible but must be justified in the application. If the simulated-use test passed but the suspension test did not pass, the applicant needs to justify why the concentration used in the simulated-use tests should be considered as the effective dose.

5.4.5.4 Disinfection in collective drinking water systems

5.4.5.4.1 Introduction

This is disinfection in collective drinking water systems like hospitals and other healthcare facilities, hotels, penitentiary institutions, etc. In these large plumbing systems water might become contaminated with *Legionella* spp. In addition to physical techniques (heating, UV treatment, etc.) chemical disinfection is sometimes allowed in some EU countries.

5.4.5.4.2 Data requirements

Test methods

For product authorisation of drinking water disinfectants in collective drinking water systems, the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred.

The following requirements are set for biocides to be used as disinfectant in collective drinking water systems:

Laboratory tests

The basic efficacy of the product should be demonstrated in suspension tests (phase 2, step 1).

Studies should show that the product can accomplish a lg reduction of 5 against bacteria and a lg reduction of 4 against *Legionella pneumophila* specifically. This can be done in laboratory tests (e.g. suspension tests EN 1276 and EN 13623). Tests should be modified to reflect the use conditions with respect to soiling, temperature range and contact time (see Appendix 4).

The suspension tests can be waived when simulated-use tests or field trials are available in which the concentration of *Legionella* spp. is high enough to show lg reduction of 5 (min. 10^5 cfu/L).

Simulated-use tests

A simulated-use test should be performed but is only mandatory in cases where a lg reduction of 4 cannot be demonstrated in a field trial due to low levels of *Legionella* spp. in the drinking water or in the suspension test.

A detailed description for a simulated-use test is given in the test method "Quantitative determination of the efficacy of drinking water disinfectants". Currently, this test can only be performed in Germany. Alternative methods will be considered and are acceptable provided they are scientifically justified: they will be evaluated by the CA on a case-by-case basis. If

this test cannot be used according to the scope of the test an alternative method can be presented. CAs will examine the eligibility of the proposed alternative. As the test method "Quantitative determination of the efficacy of drinking water disinfectants" does not cover *Legionella* spp., an experimental method to simulate a system with hot water is given in the following publications: "Development of a pilot-scale 1 for Legionella elimination in biofilm in hot water network: heat shock treatment evaluation" and "Chemical disinfection of Legionella in hot water system biofilm: A pilot-scale 1 study".

Field trials

Field trials (historic and in-use monitoring) should always be provided especially for products with long and continuous use. See below under Test Conditions/Field Trials for further details.

Test organisms

PT5 products for collective drinking water systems should be at least sufficiently effective against bacteria and specifically against *Legionella* spp. Since the control of *Legionella* spp. in collective drinking water systems is of major importance, efficacy against *Legionella* spp. (field trials) and *Legionella pneumophila* (suspension tests or simulated-use tests) should always be demonstrated in addition to general tests against bacteria.

Efficacy tests with these organisms should always be provided.

For all other groups of organisms, data only need to be provided when an efficacy against those organisms is claimed.

Test conditions

Laboratory tests

It is important that the efficacy tests are carried out with the contact time as claimed in the SPC, and also that the claimed contact time is a realistic value.

Suspension tests may be modified considering the type of disinfectant application and considering especially that the test needs to be performed reflecting the worst-case conditions (temperature, soiling, mineralization, pH). Further details can be taken from Appendix 4. For suspension tests, the maximum contact time is 25 min.

Since the water treated in collective drinking water system is clean water coming from a drinking company, laboratory tests should be carried out with soiling for clean conditions as defined in Appendix 4.

Simulated-use tests

The tests are carried out with the standard contact time (10 and 25 minutes) or as claimed in the SPC. Tests should be carried out with soiling for clean conditions as defined in Appendix 4.

Field Trials

LOCATIONS

A field trial should be performed at a minimum of 3 locations.

The drinking water quality in the different EU countries may differ. In some EU countries, disinfectants like chlorine are included as a standard, whilst in other countries, disinfectants are only added during outbreaks of pathogens. Therefore some EU countries will only accept field trials carried out within their own country or in locations with comparable water specifications. In general, however, tests are not performed in all EU countries. Therefore, in

all field trials, the quality of the tested drinking water should be clearly specified and documented. The comparability of this water to the drinking water in each country should be clearly described and justified, accordingly. Ultimately, the Competent Authority will decide whether the test is acceptable or not.

Only locations with 100 or more operational draw-off points (downstream of the application spot) are acceptable. A location is a collective drinking water system which is treated by the product. Also a part of a collective drinking water system, for instance, a wing of a building or only the cold water system, can be seen a test location as long as it contains 100 or more operational draw-off points.

DURATION OF THE TEST

When the apparatus is in continuous or discontinuous use (so no single applications) the duration of the test is one year per location, starting from the first sampling round after starting the apparatus. When, due to starting problems etc., the first months do not give the required result, the test should be extended to ensure duration of one year starting from the point at which a stable situation is reached. In this way, at least a year of test results can show that the product is capable of controlling *Legionella* spp.

DIFFERENT TYPES OF WATER

It is recommended that the locations are spread over the country, this is to ensure that the product is tested on different types of water (hardness, organic material, etc.). For this purpose, information should be provided on the quality of the provided water at the different locations. In principal this information is available through the water suppliers.

LEGIONELLA

Before starting a test it should be clear that the installation to be treated is contaminated with *Legionella* spp. bacteria (≥1000 cfu/l). For this purpose, information should be provided on (recent) problems with *Legionella* spp., like results from sampling in the past and performed cleanings, etc. The system should not be artificially contaminated.

SAMPLING POINTS

The amount of sampling points per location depends on the amount of draw-off points (taps and other outlets) in the installation. The table below should be used.

Table 13: Number of sampling points

Number of draw-off points (outlets)	Number of sampling points
101 – 200	6
201 – 400	8
401 – 800	10
801 – 1600	12
> 1600	14

All sampling points should be unambiguously coded.

At each sampling round two sampling points are sampled each time (standard sampling points), preferably the sampling point next to the apparatus and the sampling point the

most far away from it. These sampling points should be clearly described and the code of these points should be stated. All other sampling points may vary at each sampling round. When a sampling point shows elevated values of *Legionella* spp., or one of the other parameters, this sampling point should be sampled again the next month. The total amount of sampling points should remain the same, according to the table above.

The tuning of the apparatus from which the disinfectant is dosed should be recorded at the time of sampling.

EFFICACY

The following measurements should be performed:

- zero measurement: measurement of *Legionella* spp., total hardness, pH, organic contamination of the water and residues of active substances from previous treatments before the disinfection treatment is started;
- *Legionella* spp., monthly sampling, norm value 100 cfu/l (90%-percentile with a maximum of 1000 cfu/l);
- total hardness, Ca, Mg; sampling once per four months, depending on the variation a higher frequency might be necessary; also data from the water supplying companies can be collected;
- pH, monthly sampling on both standard sampling points, or data from the water supplying companies can be collected.

ACTIVE SUBSTANCES

To determine the amount of active substance in the water, the relevant substances should be measured monthly.

In general, the active substance of the used biocidal product should be measured monthly. The sampling point as stated in Table 13 of this section should be taken. Especially the first and the most far away sampling point are of importance, in order to ensure that enough product reaches the end of the system. These data are especially relevant for the efficacy assessment of in situ generated products and can also be used in other areas (e.g. for toxicological and environmental risk assessment).

GENERAL REQUIREMENTS FOR STUDY REPORTS

Every study report should contain a good description of the material (location, number of draw-off points, sampling points, history of *Legionella*, etc.), the method (starting date, tuning of the apparatus from which the disinfectant is dosed) and the results (including 0-measurement). In the study reports of the field trials, the results should be interpreted per location. Remarks such as high values above the norm, should be mentioned and explained. The report should contain a conclusion.

APPARATUS

In case of *in situ* production of the active substance or when an apparatus is used to dose the active substance in the right amount to the water, the report should contain information on safety measurements concerning over and underdosing. Continuous measurement of the dosed active substance should be established. The devices used to generate the active substance *in situ* themselves are not covered by the provision of BPR and consequently are not subject to the authorisation.

Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory, simulated-use and field trials have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT5 products the required lg reductions in suspension tests are referenced in Appendix 4.

The pass criteria for the simulated-use test are stated in the test (see Appendix 4).

For the evaluation of the results of the measurements in the field trial, the norm values used are mentioned above under Test Conditions/Field Trials. Per location, 90% of the measurements should fulfil the requirements. Over all locations together, 90% of the locations should fulfil the requirements.

Deviations from the pass criteria are possible, however, they must be justified in the application.

5.4.5.5 Disinfection of water in reservoirs

5.4.5.5.1 Introduction

This is disinfection of water stored in tanks and reservoirs, for instance on ships, mobile homes, or in small tanks as in a dentist's chair. It is presumed that these tanks start filled with water of drinking water quality. The disinfection product should maintain the quality of the water over time. When the product is also intended to disinfect water from other sources (e.g. groundwater, spring or surface water) this should be clear in the claim for the product. It should also be specified whether the tank should be cleaned before disinfection or not. The claimed use should be specified in the SPC.

5.4.5.5.2 Data requirements

Test methods

For product authorisation of drinking water disinfectants in reservoirs the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred. Next to a suspension test a simulated-use test should be performed.

For suspension tests EN phase 2, step 1 tests are preferred. Since for most target organisms there are no specific EN tests for drinking water disinfection, tests should be modified to reflect the use conditions with respect to soiling, temperature range and contact time. EN tests from food and industrial area (see EN 14885) can be modified (see 'Test conditions' below). For virucidal activity EN 14476 can be modified. Efficacy suspension tests should be provided with two concentrations: the concentration of the product as dosed (start concentration) and the active substance concentration obtained in the field at the end of the claimed period of use.

For disinfection of water in reservoirs it is mandatory to provide a simulated-use test. Such tests are required in order to demonstrate the proper distribution of the disinfectant in the reservoir. In the absence of a standard method, the applicant should provide a testing proposal which needs to be agreed by the CA in advance. Alternatively, for products with one active substance that can easily be measured, efficacy can be demonstrated using a field trial in which the amount of active substance and the amount of organisms is measured several times during the test period.

In some cases, efficacy against biofilm is of importance in this use. For testing efficacy against biofilms see section 5.4.2.11 of this Guidance.

In cases when water is of drinking water quality in the beginning and the disinfectant is used to maintain water quality, the information should be provided on how long the effect can be guaranteed at a certain temperature and a maximum DOC. This should be justified and demonstrated in the efficacy tests.

For an overview of available EN tests see Appendix 2.

Test organisms

Drinking water disinfectants for reservoir water should be at least sufficiently effective against bacteria and viruses. Tests with these organisms should always be provided.

For all other groups of organisms (e.g. *Legionella* spp. or Protozoa) tests only have to be provided when efficacy against these organisms are claimed.

The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference test organisms for PT5 is given in Appendix 3.

Test conditions

It is important that the tests are carried out with the same contact time as claimed in the SPC. The claimed contact time has to be a realistic value. Therefore, the applicant has to clearly indicate how long the disinfectant can guarantee the quality of the water in the reservoir. When started with raw water it should be indicated at what time after the treatment the water can be used.

If protozoa are claimed, tests with longer contact times relevant for protozoa are acceptable.

When starting with water of drinking water quality, tests should be carried out with soiling for clean conditions as stated in Appendix 4. For this type of product (if tested under clean conditions), the applicant needs to clearly indicate to the user that the reservoir should be clean before filling it with fresh and clean water.

When starting with raw water, tests should be carried out with soiling for dirty conditions in accordance with the test requirements (see Appendix 4).

Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and simulated-use tests have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. The required lg reductions in suspension tests are referenced in Appendix 4.

Deviations from the pass criteria are possible, however, they must be justified in the application.

5.4.5.6 Disinfection of water of undefined quality for small scale use (up to 5l/person/day)

5.4.5.6.1 Introduction

This is disinfection of, for instance, individual emergency water supply or other water that might be contaminated in places where no clean drinking water is available. This means water not originally coming from the drinking water suppliers. This is only intended for water that is used directly for drinking or preparing food after disinfection, therefore for small scale use (up to 5 L/person/day).

5.4.5.6.2 Data requirements

Test methods

For this use it is in most cases acceptable to demonstrate efficacy in a suspension test only. For suspension tests EN phase 2, step 1 tests are preferred. Since for most target organisms there are no specific EN tests for drinking water disinfection, tests should be modified to reflect the use conditions with respect to soiling, temperature range and contact time. EN tests from food and industrial area (see EN 14885) can be modified (see 'Test conditions' on the next page). For virucidal activity EN 14476 can be modified. For an overview of available EN tests see Appendix 2.

If due to turbidity a pre-treatment is needed, such as filtration, this should be part of the test conditions. It is the responsibility of the applicant to clearly instruct that a pre-treatment is required due to turbidity. This should also be reflected on the SPC of the product in the section "Instructions of use" together with the exact treatment duration.

If no pre-treatment for turbidity is involved for turbid water, the field trials should be performed. Field trials should be performed with different raw water (mineralisation, TOC, temperature, pH) in which turbidity is considered.

Test organisms

Drinking water disinfectants of "water with undefined quality (small-scale use)" should be at least sufficiently effective against bacteria and viruses. For all other groups of organisms tests only have to be provided when efficacy against the organisms that are claimed. The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference test organisms is given in Appendix 3.

Test conditions

A suspension test needs to be performed reflecting worst-case conditions (temperature, soiling, contact time, mineralization, pH). The test should be done with the claimed contact time but no longer than 30 minutes. The suspension test (EN phase 2, step 1 – food area) should be carried out with soiling for dirty conditions (see Appendix 4).

For the field trial, at least three types of raw water should be tested. Information on mineralisation, TOC, temperature, pH and turbidity should be given.

Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory and field trials have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT5 products the required lg reductions in suspension tests are referenced in Appendix 4.

Deviations from the pass criteria are possible, however, they must be justified in the application.

5.4.5.7 Disinfection of water for animals

5.4.5.7.1 Introduction

This is disinfection of water in animal housing used as drinking water for animals and for other uses in animal houses (preparing feed, etc.). When products are used to disinfect water for both humans and animals, requirements according to sections 5.4.5.2 to 5.4.5.4

are also applicable. The origin of the water in water systems for animals can differ, e.g. groundwater, surface water (dirty), or water from drinking water suppliers (clean). The intended use should be specified on the SPC.

5.4.5.7.2 Data requirements

Test methods

For efficacy testing of disinfectants for water for animals the tiered approach as described in section 5.4.0.4.1 of this Guidance is preferred. Next to a suspension test also a simulated-use test or a field trial (phase 3) should be performed, to provide information under in-use conditions. In some cases, efficacy against biofilm is of importance in this use. For testing efficacy against biofilms see section 5.4.2.11 of this Guidance. For suspension tests EN phase 2, step 1 tests are preferred. Since for most target organisms there are no specific EN tests for drinking water disinfection, tests should be modified to reflect the use conditions with respect to soiling, temperature range and contact time. EN tests from food and industrial area (see EN 14885) can be modified (see 'Test conditions'). For virucidal activity EN 14476 can be modified.

For the simulated-use test a detailed appropriate test method is given in the test method "Quantitative determination of the efficacy of drinking water disinfectants". The test is realised on an adapted test rig. A disinfectant neutralizer or filter system to stop a reaction between disinfectant and test organisms is required. Currently, this test can only be performed in Germany. Alternative methods will be considered and are acceptable provided they are scientifically justified and will be evaluated by the CA on a case-by-case basis.

Since drinking water for animals can be obtained from a variety of different sources, e.g. surface water (lakes, rivers), underground water pumped from wells, human drinking water, rainwater, etc., several kinds of water should be tested. Alternatively, it should be indicated on the label under which conditions the product can be used.

Test organisms

Drinking water disinfectants of water for animals should be at least sufficiently effective against bacteria. For all other groups of organisms tests only have to be provided when efficacy against the organisms are claimed. The test organisms used in efficacy tests are normally stated in the applicable standard test methods. An overview of reference test organisms is given in Appendix 3.

Test conditions

It is important that the efficacy tests are carried out with the contact time as claimed in the SPC, and also that the claimed contact time is a realistic value.

Suspension tests may be modified considering the type of disinfectant application and considering especially that the test needs to be performed reflecting the worst-case conditions (temperature, soiling, contact time, mineralization, pH). Further details can be taken from Appendix 4.

Laboratory tests should be carried out with soiling for clean or dirty conditions as defined in Appendix 4. Depending on the water source that has to be disinfected the test should be performed under either clean or dirty (e.g. undefined or pumped up water) conditions.

Field trials should be done in animal housing. A testing proposal needs to be provided taking into consideration relevant parameters, such as type of water to be treated (e.g. water originating from the public distribution system or surface water), pre-cleaning of the

"distribution system", pre-treatment of the water (e.g. physical treatment such as filtration) and application of food additives or antibiotics which will be evaluated by the CA on a case-by-case basis.

Acceptance criteria

A product will be assessed to be sufficiently effective if the required laboratory tests, or when applicable, field trials have been carried out (using the required test organisms and test conditions), and when the pass criteria for the tests have been met.

Where pass criteria are available in the standard test these should be met. For PT5 products the required lg reductions in suspension tests are referenced in Appendix 4.

The pass criteria for the simulated-use test are stated in the test (see Appendix 2). Field trials should demonstrate sufficient efficacy and the microbiological burden should stay below an acceptable level according to the relevant legislation. Deviations from the pass criteria are possible, however, they must be justified in the application.

5.4.6 Materials and Articles Treated to Protect Humans or Animals

For testing materials and articles with claims to protect humans or animals, a tailored approach is compulsory. The testing strategy entirely depends on the specific claim made. In the majority of cases, a claim can only be made for a specific type of final article, as use area and use conditions are decisive for describing the problem which the biocide must solve, and to demonstrate efficacy in exactly those conditions is necessary. Consequently, this section describes testing principles and strategies rather than recommending specific tests.

A tiered approach has to be followed in demonstrating claims for protection of humans or animals:

- Tier 1 - Proof of principle: tier 1 tests should document the efficacy of the incorporated biocide in the relevant matrix against relevant target organism(s) under relevant conditions, e.g. humidity, temperature.
- Tier 2 - Simulated Use: tier 2 tests should document the efficacy of the incorporated biocide in the relevant matrix under real-life conditions, e.g. way of contamination, cleaning regimes, time to take effect and the duration of the effect.

Depending on the claim made, e.g. "kills bacteria on door-handles to prevent cross contamination", "protects against mosquito-bites", even tier 3 testing can be necessary:

- Tier 3 - In-Use Evaluation/Field studies: To substantiate health benefit claims, treated and untreated articles would be tested via statistically designed use trials by a representative user group.

Generally, the principle applies that only claims can be made which have been demonstrated.

5.4.6.1 Determining the purpose of the Treatment

The effects of articles with a disinfection claim cannot be detected by changes in appearance, mechanical properties or odour. The precondition for demonstrating efficacy is a clear description of the purpose of the treatment. Often, claims are unclear about whether the treatment prevents growth or kills bacteria on contact. On most articles, no bacteria will grow under normal conditions of use. Nevertheless, antibacterial claims (such as 'anti-bacterial', 'hygienically clean', 'free of bacteria', 'prevents the spread of hazardous bacteria')

are made, insinuating that bacteria will be killed on the material, though only growth inhibition tests have been carried out. In most environments, the sheer presence of bacteria does not present a problem. If this is a problem, it is in most cases much more effective to use traditional disinfection methods with a liquid disinfectant. In most cases, the treatment of articles should not be used as the only measure of disinfection but should be combined with a disinfection management regime.

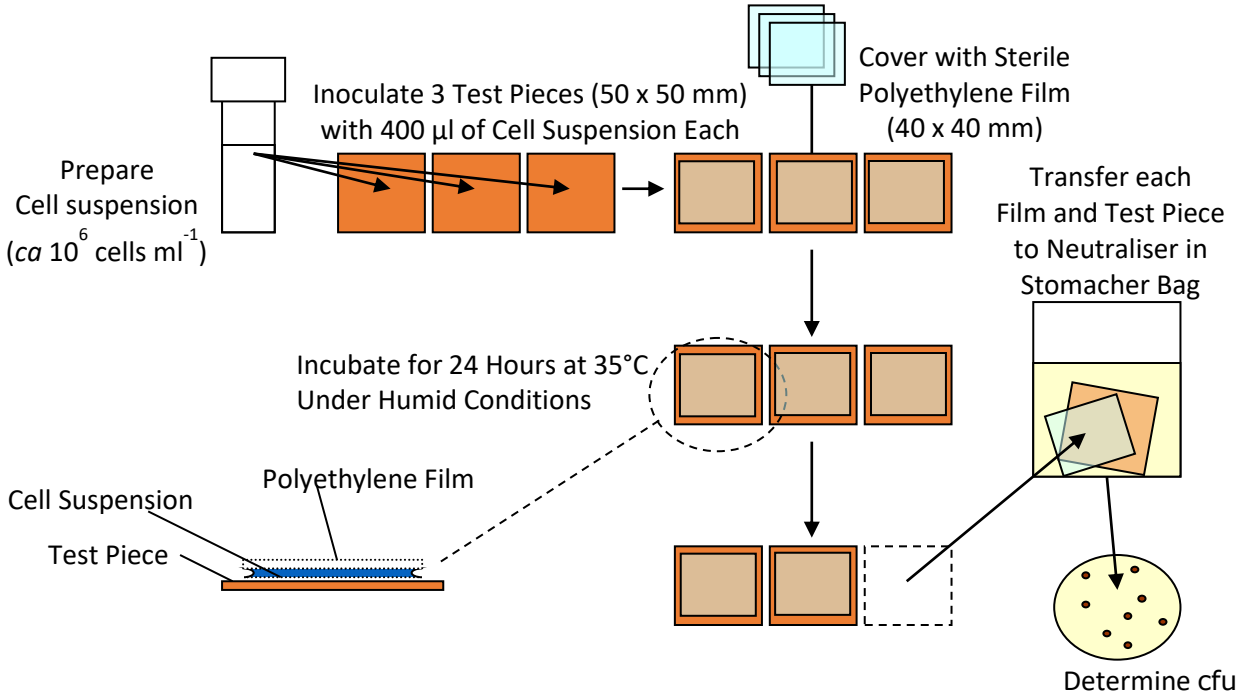
5.4.6.2 Effects Intended to Inhibit Microbial Growth

Under the majority of indoor situations, most micro-organisms will not grow on environmental surfaces due to lack of humidity. To make a claim for growth inhibition, wet or at least humid conditions are a precondition, unless otherwise justified. To demonstrate such a claim, sub-samples of treated and untreated material of the article in question could be tested using a method adapted from ISO 22196 (see Figure 3). Soiling conditions, temperature, test species and contact time have to be adapted to mimic a realistic in-use situation (tier 1). The impact of in-use conditions like ageing or cleaning regimes on the effect would have to be included in the testing (tier 2). The minimum requirements for disinfection are laid down in the Claims matrix for treated articles (Appendix 1[22]) with claims to protect humans or animals.

[22] See Appendix 1: Claims matrices for PT 1-4 and treated articles

Figure 3: A Test for Antibacterial Activity in Wet Conditions

ISO 22196



ISO 22196, Method Outline:

An aliquot (usually 400 µl) of a log phase bacterial cell suspension (*ca*10^5 cells ml^{-1}) in $^1/_{500}$ Nutrient Broth are held in intimate contact with each of 3 replicates of both treated and untreated variants of the test materials using a 40 mm x 40 mm polyethylene film (*e.g.* cut from a sterile Stomacher bag) for 24 hours at 35°C. Usually, *S. aureus*, *P. aeruginosa*, *Enterococcus hirae* and *E. coli* should be tested (see Appendix 3). The populations are then recovered using a neutraliser solution and the size of the surviving populations are determined as colony forming units using a dilution plate count method. Additional replicate unfortified samples are also inoculated in the same manner but are analysed immediately to determine the size of microbial population present prior to incubation. The differences between the initial and final population as well as between the treated and untreated materials are used to assess the basic antibacterial properties of the test materials.

5.4.6.3 Effects intended to Kill Micro-organisms through Contact

Claims made for materials and articles to kill on contact to prevent cross-contamination are not easy to demonstrate. Mostly, the effect will require the release of the active substance from the surface of the material; this release needs to be triggered somehow. In the majority of cases, water or other liquids are the crucial component to facilitate such release and transfer. If the event that caused the deposition of the target organism does not introduce moisture and the normal exposure conditions of the material or article are dry (or only subject to normal, ambient indoor humidity), the effect of the treatment will probably be limited.

Another issue is the speed of activity needed to inhibit cross-contamination. If for instance door handles in a hospital would be treated with an active substance to kill deposited pathogenic organisms, the effect would have to be sufficiently fast to prevent the next

person using the door handle from cross-contamination. In combination with the little moisture which is deposited in the event, it will be challenging to demonstrate a satisfying effect. The minimum requirements for disinfection are laid down in the Claims matrix for treated articles (see Appendix 1) with a claim to protect humans or animals. Additional requirements may apply depending on the claim made.

Testing could be carried out using protocols such as those given in Figures 4, 5 and 6 below. Again, care must be taken to adapt test conditions to realistic in-use conditions. Figures 4 and 5 show the approach used for non-porous materials and for absorbent materials, respectively, both intended to simulate contamination through contact with splashes of contaminated liquids. Figure 6 illustrates a protocol intended to simulate contamination through, for example, hand/gloved hand contact.

5.4.6.4 Acceptance Criteria

The performance criteria for treated articles can be found in the Claims Matrix for treated articles (Appendix 1). For choosing test organisms please refer to the liquid disinfectants (Appendix 3). As the performance criteria for treated articles are lower than for liquid disinfectants, the treatment of articles should generally not be used as the only measure of disinfection, but should be combined with a disinfection management regime.

Figure 4: Simulated Splash Model Non-Porous Materials

cfu= colony forming units

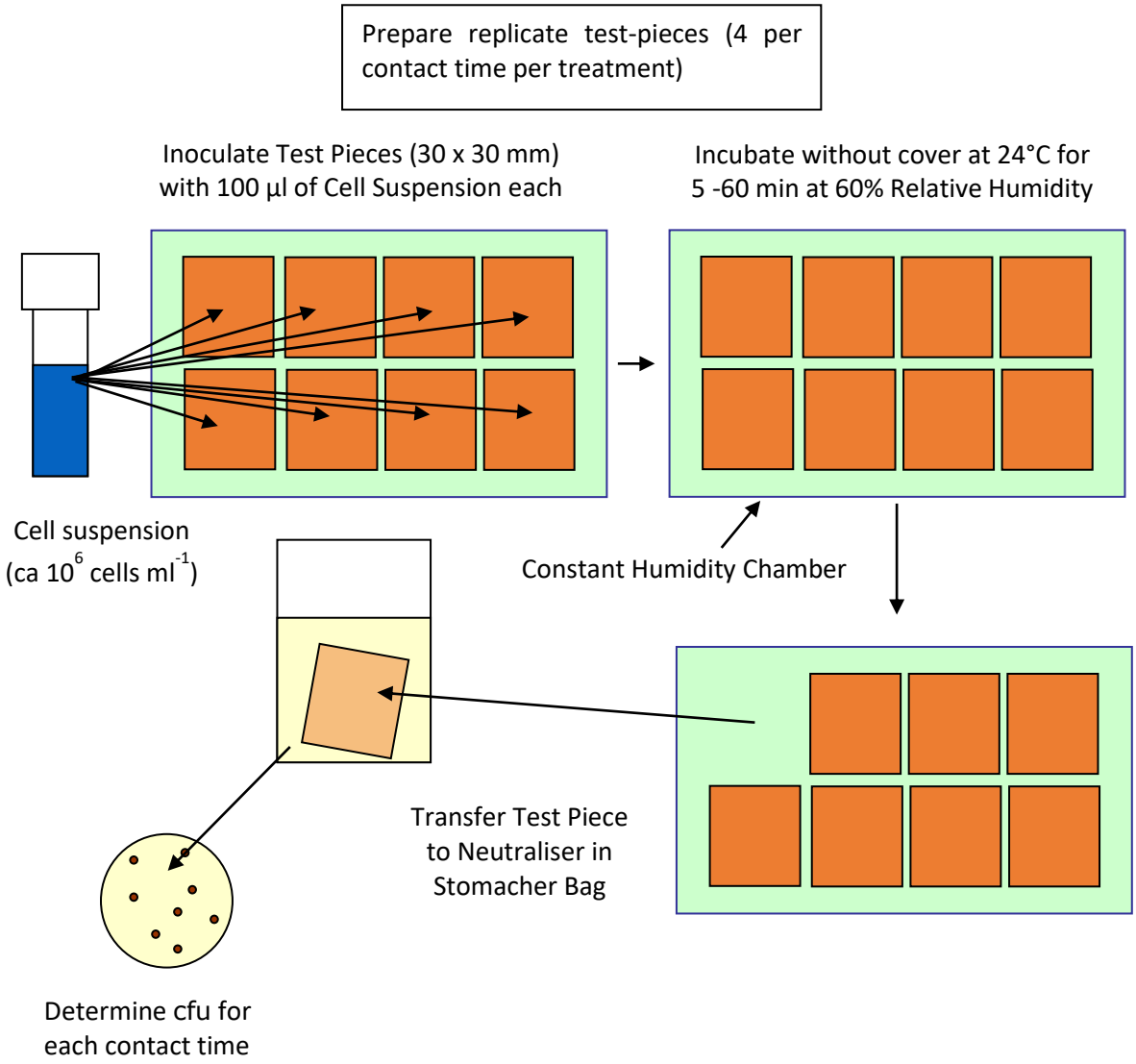


Figure 5: Simulated Splash Model Porous Materials

cfu= colony forming units, RH= relative humidity,
BSA= Bovine Serum Albumin

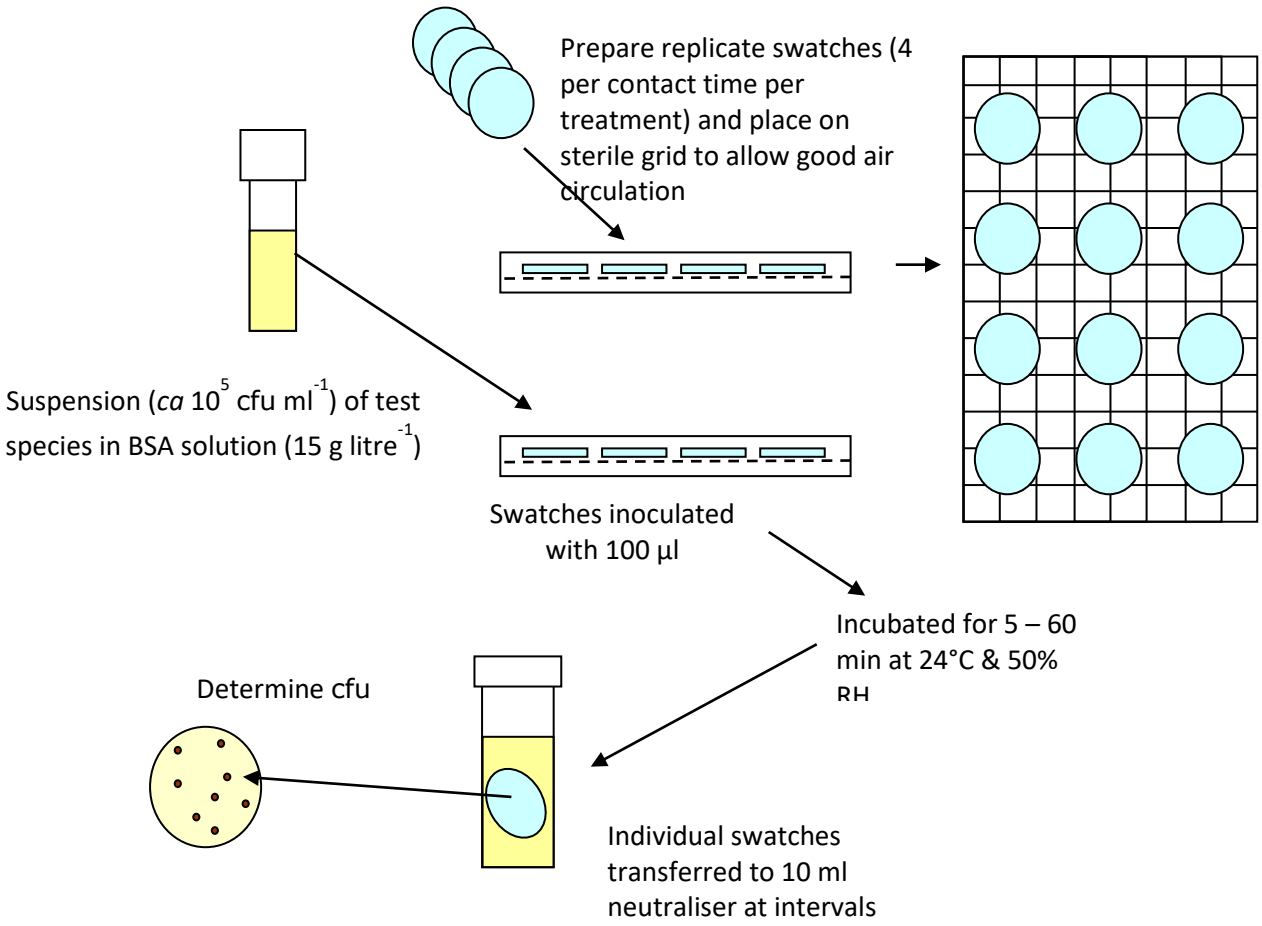


Figure 6: Printing Model

TVC= total viable count
cfu= colony forming units

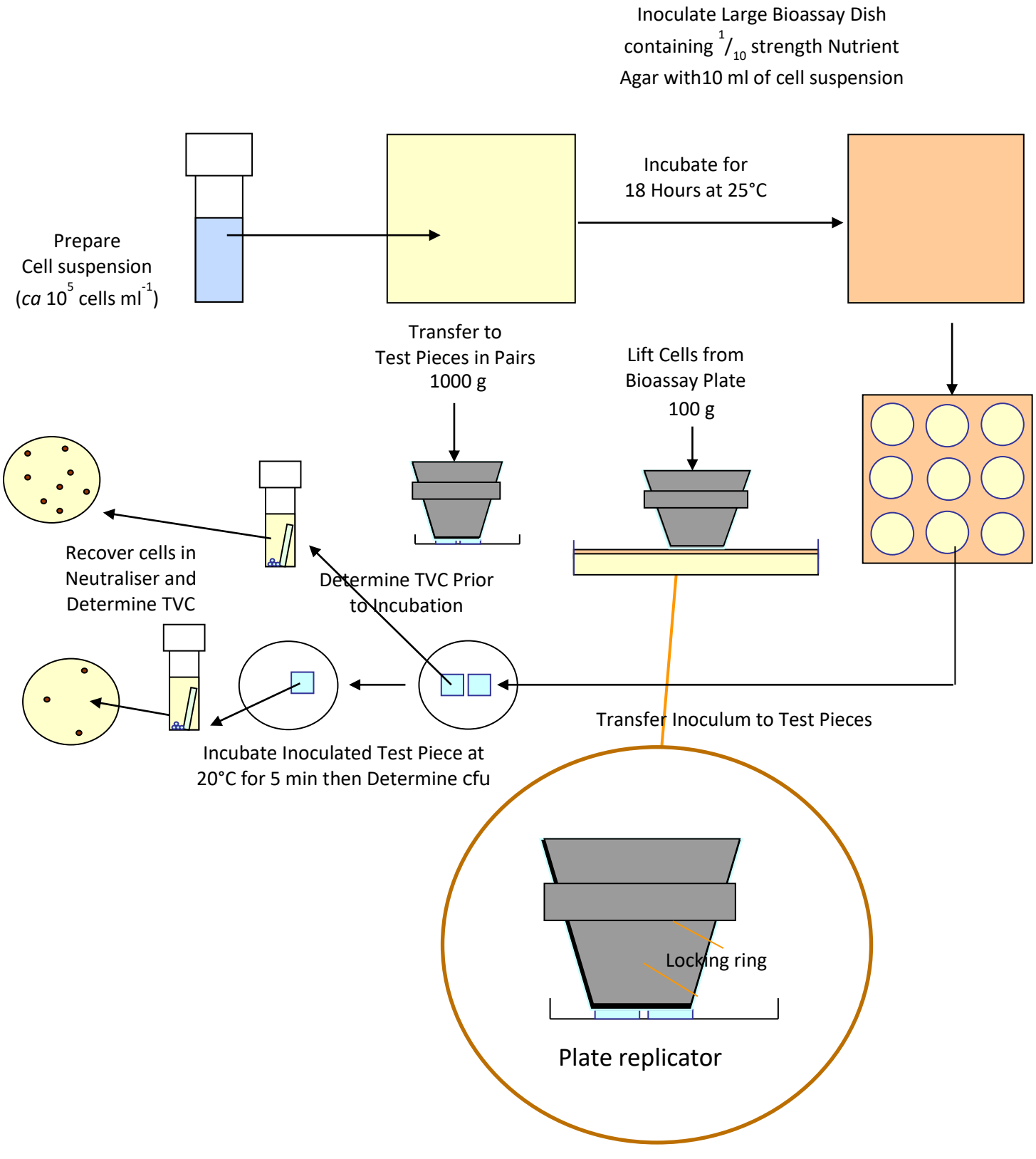


Table 14: Protection of Humans or Animals – Example Claims, Problems and Testing Approaches

Claim	PT	Proof required	Example method
Bedside cabinet for use in hospitals that has been treated to reduce infections by killing 'bacteria on contact'.	2	Data should show that micro-organisms, when deposited through skin contact (even under simulated conditions) and through the deposition of fine aerosols are killed within a time-frame that would prevent the surfaces becoming a vector for cross-contamination.	Plaques made of the identical material used for the cabinet are employed in the test. Both treated and untreated variants are used.
		Skin contact	The method described in Figure 6 is employed to deposit bacteria onto test plaques. A range of contact times between 5 minutes and 1 hour are used. A lg reduction of 3 should be achieved.
		Aerosol	The method described in Figure 4 is adapted for use by employing multiple droplets of 1 µl on each test plaque. A range of contact times between 5 minutes and 1 hour are used to explore activity. A lg reduction of 3 should be achieved.
A plastic conveyer belt is treated to prevent the growth of bacteria between cleaning intervals in a food factory.	4	Data should show that relevant bacteria grow on an untreated conveyer belt under normal conditions of use during a 6 hour interval. Significantly reduced growth should be demonstrated on the treated belt.	Plaques made of the identical material used for the belt are employed in the test. Both treated and untreated variants are used. ISO 22196 is adapted to simulate a moist conveyor belt. A soiling agent relevant to the end use is included. A contact time and temperature equal to that encountered in practice are employed.

Table 15: Basic Requirements for a Valid Test Protection of Humans or Animals

The following summary provides a guide to the basic requirements for a valid test:

i. The test should be carried out on the type of final article.
ii. A test which mimics the way of deposition and the type of material needs to be chosen.
iii. An untreated variant of the test material must be included such that the impact of the treatment can be demonstrated.
iv. Test conditions should reflect normal conditions of use in terms of humidity, temperature, soiling, contact frequency, etc.
v. The test should employ organisms that are relevant to the end use of the article and the purpose being claimed.
vi. Tests that employ a single species of organisms should be favoured over those that use consortia.
vii. Minimum of three replicate test pieces of both treated and untreated materials should be employed (unless justified).
viii. The final data should include either some indication of the impact of service conditions on the performance of the treated material/article or data from an ageing study. The intention is to demonstrate how long the claimed effect will be sustained.
ix. If claims are made which require a field trial, relevant data including statistical evaluations have to be provided.

5.5 Preservatives (Main group 2)

General

Preservatives in main group 2 are intended to prevent the biodeterioration of a material or a matrix. Wood can lose stability by the action of micro-organisms or insects, fabric can be destroyed by fungi, and even polymer-based plastics are prone to biological deterioration. Plasticised PVC would soon become fouled by surface growths of fungi, lose plasticity and crack without the inclusion of a fungicide. A water-based paint, free of volatile organic compounds (VOCs), could not be stored without the use of a biocide. Polyurethane, for example as used for the soles of shoes, can become colonised by fungi and actinomycetes. The heat exchangers in cooling towers have to be kept free from microbial growth to enhance performance by treatment of the cooling liquid.

This section covers the group of preservatives (PT6 to PT13) and the following sections (5.5.1-5.5.3) apply to all PTs (or as indicated in the headings). For PT8, the guidance is more developed and includes standard tests, which is not the case for the other PTs: PT8 is the exception and section 5.5.8 is dedicated to PT8.

5.5.1 Distinction between preservation/curative treatment and disinfection

Preservatives are directed towards the protection of a *material*. If the material itself is not affected by the target organisms, the claim does not belong in main group 2. The aim of preservation is to prevent microbial spoilage, decay or the accumulation of biomass that is detrimental to the functionality of an item, material or system. Detrimental effects can be caused by proliferation of cells or by the metabolic activity of cells and may not necessarily involve cell multiplication. The presence of micro-organisms can result in either a degradation of the matrix in which they are present or damage to the system in which they are present either due to their metabolic activities (e.g. corrosion) or by fouling or blocking pipes, forming biofilms on heat exchangers etc. It is not the intention of preservatives to transfer their effects to other materials, humans or animals, but to protect the material itself. A long-term effect is generally required. A preservative can have a reversible effect on micro-organisms (e.g. by causing stress or cell damage without total loss of viability). In contrast to disinfection no level of reduction is defined for a set of predefined claims.

Curative treatments are also directed towards material protection and therefore likewise fall into main group 2[23]. The aim of a curative action is to either cure microbial spoilage which has already occurred or to eliminate / reduce populations in materials and systems prior to them being treated with a preservative (in some instances a biocidal product can have both curative and preservative functionality).

The level required to prevent spoilage in different media/conditions will be defined by the individual claim made. This will also be the case when the treatment is intended to achieve a curative action.

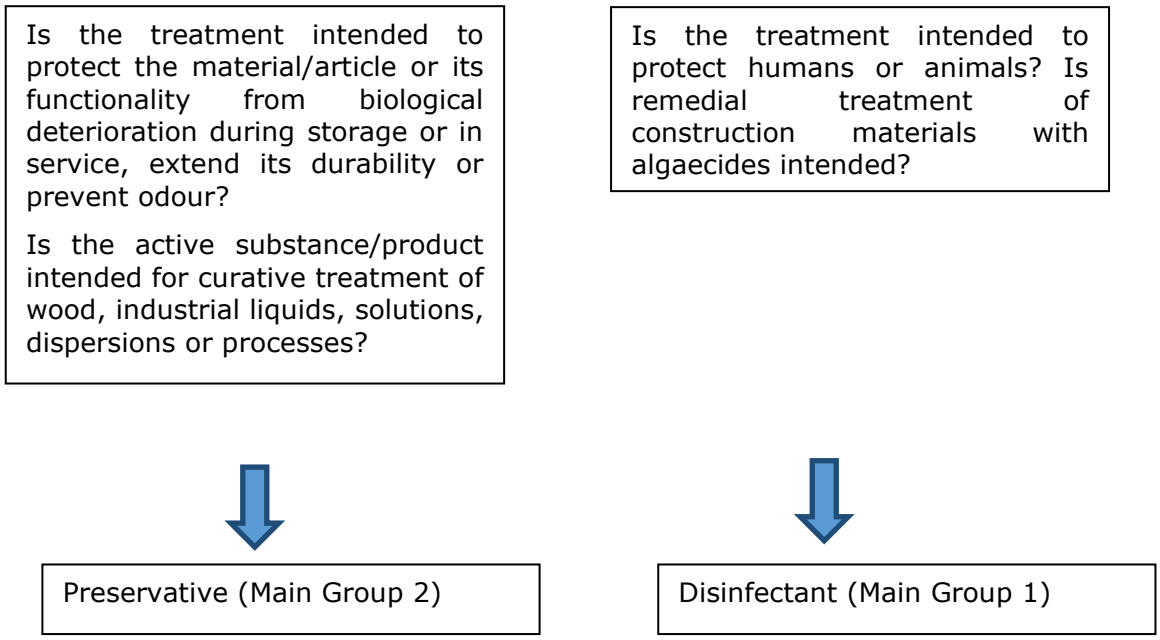
The claim made will define in which of the PTs an application will fall. The following data is needed:

- A problem description: Scale, speed and type of effect required and what would happen if the biocide was not present
- The target organisms

[23] See CA-Sept15-Doc.8.3 – Curative use of preservatives

- Categorisation of the material/matrix to be treated including dose-rate/concentration of the biocide in the material/matrix.
- The intended use pattern of the treated material/matrix including service-life, weathering conditions, leaching (intended or unintended).

Figure 7: Decision scheme for the distinction between preservation/curative action and disinfection



5.5.1.1 Curative uses

Curative uses often require rates and speeds of effects that are similar to those required for disinfectants but do not have prescribed performance standards (with the exception of some PT8 standards). Such uses are nevertheless intended to cure (eliminate or reduce) contamination in materials, matrices or systems. They therefore fall under main group 2. Performance requirements will be defined by the requirements of either the matrix or the process involved. A curative effect and a preservative effect may sometimes be achieved using the same biocidal product, only the concentration may differ. In other cases active substances with curative properties will be combined with those that have preservative. Curative and preservative effects need to be demonstrated separately and different methods need to be employed. When claims are made for curative uses, it is important to carry out the health and environmental risk assessment with any higher doses that may be required. A typical example of curative action is the treatment of a contaminated product prior to packaging and sale (in some cases in addition to a preservative – in other cases the curative product may be capable of achieving both a preservative and a curative effect). Another example is the treatment of a contaminated system by reducing the microbial population it contains to limits that are acceptable to the process (e.g. on a paper mill). Please read more about testing of curative uses in section 5.5.5.1 and 5.5.8.

5.5.1.2 Borderline case: Algaecides

If algae are expected not to destroy the material or damage its function, algaecides are not considered to be preservatives. Thus products used against algae for treatment of swimming pools, aquariums and other waters and for the *remedial* treatment of construction materials belong to product type 2 in main group 1, whereas products with *protective* function are considered as products belonging to main group 2.

For example surface coatings for outdoor use are often formulated with both a fungicide and an algaecide. The algaecide, like the fungicide, is performing a preservative function in the coating and is thus covered by PT 7. Similarly, algaecides are incorporated into plastics (e.g. electricity pylon insulation sleeves - to prevent growth that would otherwise cause arcing and system failure) and material used in aquatic and marine environments (including some cementitious materials). Algae are a problem in many water-based cooling systems and water-based process systems (e.g. paper making), where either a preservative or a curative action may be required. Such applications likewise belong to main group 2.

5.5.1.3 Borderline cases: Treated articles

Treated articles can both belong to Main Group 2 or Main Group 1 (and even to Main Group 3 or 4). Please refer to section 5. 3 on treated articles and section 5.4.6 on materials and articles treated to protect humans or animals.

5.5.2 Principles for testing preservatives

The aim of any preservation is to maintain the present state/properties of a material or matrix along with it its functionality. This can be done in several ways: To determine microbial activity in a biocide-free material, the method of measuring colony forming units is the most common approach to prove that a preservative is needed, i.e. the population needs to be shown to increase in size in the untreated material. The production of a biofilm or an increase in biomass may also be appropriate. Other parameters indicating metabolism can also be documented like e.g. changes in pH, in viscosity, in colour. Data needs to be recorded from the beginning of the test (incubation time 0) and before and after each new inoculation.

Showing growth / metabolism of the micro-organisms in the untreated system is an essential requirement of any demonstration of effectiveness of an active substance or biocidal product. It is then assumed, if not proven in every case, that changes have taken place that were induced by microbial growth and that this can be prevented by the use of a biocide acting as a preservative. Often, when growth cannot be proven this is caused by an unnecessarily high inoculation rate. If, at the beginning of the test, an inoculum of for example 10^4 cfu for bacteria is employed, an increase to 10^5 - 10^6 can often easily be shown during the test period. When a higher inoculum density for example 10^6 is employed, growth is much harder to achieve due to limitations in the supply of nutrient etc. An important consideration is to use a model substrate that can support growth readily rather than attempt to achieve growth in a final product that is less susceptible to the non-acclimated species employed in laboratory tests (i.e. it is often nearly impossible to replicate the failure phenomena observed in practice in a laboratory).

Often a fungicidal or bactericidal claim needs to be supported. For this purpose a species can be tested singly or, as it is good practice in many test protocols, in mixed suspensions of either bacterial species or fungal species. Mixing of bacteria and fungi should generally be avoided in these suspensions, but filamentous fungi (“moulds”) and non-filamentous fungi (“yeasts”) can be mixed in the inoculum. However, for determining growth different methods need to be applied for yeasts and filamentous fungi.

Many micro-organisms are able to form dormant cells or spores to survive unfavourable environmental conditions. These resting cells do not proliferate and show no significant metabolic activity until they find a suitable environment. It is therefore possible that vegetative and active cells, being exposed to an unfavourable environment e.g. a synthetic paint containing solvent or a preservative, are forced into dormancy. Only when a sample of the material is taken out of this environment and is spread onto a nutrient medium do the cells start to grow and to build new colonies. This underlines that the appearance of colony forming units (cfu) on a nutrient media is not necessarily sufficient evidence that growth had been occurring in the matrix used in the test. Growth can only be determined by counting cfu and demonstrating that the number of cfu increased in the untreated matrix during incubation, compared to the number measured immediately after inoculation. The same or a smaller number of cfu than measured initially demonstrates survival, but not necessarily growth. However, for testing solid material, showing growth by adding a nutrient medium to the material is not necessarily enough. It needs to be shown that the material itself is damaged or loses its functionality, or, alternatively, provides growth of micro-organisms relevant for the group of organisms which have a negative impact on the stability and/or functionality of the material. Please read more in section 5.5.7.

5.5.3 Tiered approach to testing preservatives

A tiered approach should be followed for testing biocidal products:

Tier 1 - Proof of principle: tier 1 tests should document the biocidal efficacy of the incorporated biocide in a relevant model matrix against the target organism(s) under relevant basic environmental conditions (e.g. temperature, humidity).

Tier 2 - Simulated Use: The biocide should demonstrate efficacy under real life conditions relevant to its anticipated service life. Factors such as weathering, UV-stability, extended ageing or leaching should be considered.

Tier 3 – In-use evaluation/field studies: to substantiate specific claims, treated and control articles/products can be tested via statistically designed in-use trials by a representative user group, or by other appropriate methods.

In a tier 1 test, the damage should be shown in a model matrix and demonstrate how the inclusion of the biocide prevents it (often with the help of an inoculum representing the organisms that cause the damage). In a tier 2 test, damage or impact of the target organisms under either simulated-use conditions or in a manner that simulates an anticipated shelf life should be shown, and even sometimes without the use of an inoculum (soil burial). When moving up from tier 1 to tier 2, a test design has to be more tailored to the field of application envisaged. In tier 1, existing standards are often suitable when the biocide is tested in a relevant matrix with defined organisms and under relevant and reproducible conditions (which are normally only to be found in a laboratory). In tier 2, testing is more complex and often specific standards do not exist. However, sometimes the same standards can be used as for tier 1 tests, simulating use conditions by employing pre-treatment of the matrix. There may be a need for weathering cycles, wind tunnel tests, cleaning regimes etc. Similarly soiling and the influence of other micro-organisms can be of more significance. Accelerated aging tests may have to be performed before microbiological testing to allow for factors such as UV, temperature changes, leaching etc. Consideration must be given to which environmental conditions are relevant for simulated aging in realistic in-use conditions. When aging is performed in the field or under in-use conditions, reproducibility can become a difficult issue, as the aging factors such as e.g. evaporation and soiling are difficult to reproduce and can influence the results. Generally, the applicant

should be able to justify how the specific conditions used in testing relate to the in-use conditions relevant to the product or active substance. Tier 3 testing entirely depends on the claim made and is generally for specific uses in case of specific claims. The results have to be relevant for that claim and to be scientifically sound.

5.5.4 Standard Test Methods

A list of the most commonly used standard test methods can be found in Appendices 8, 9 and 10; however, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor as to when and where these tests can be meaningful to prove /support a claim and when they aren't. In contrast to disinfection, there are no specific tests allocated to the different tiers, with the exception for PT8 where standard-tests are available and tiered testing is defined, (see section 5.5.8 for more information). Often the same test can be employed for tier 1 and tier 2, and only the pre-treatment of the matrix will differ. Different factors can trigger the choice of a test: In some cases the choice of one type of method over another is related to the speed with which it generates results. Often, a method is 'known' to be capable of guiding the choice and concentration of a biocide for a certain material through experience within an industry. However, this may not necessarily mean that the method is suitable for demonstrating the claim made.

Care has to be taken as to whether the test method is appropriate for the testing of preservatives, or if it is intended to prove a curative/sanitising activity of a biocide. Generally, for preservative action growth needs to be shown in the untreated controls. The number of replicates required by the methodology is not necessarily 3 replicates; in such cases this needs to be explained and justified.

Nevertheless, an existing test method can form a good basis regarding the parameters of choice of micro-organisms, temperature, and choice of neutraliser. If necessary, these methods need to be amended by adding untreated control samples, determining the numbers of organisms that can be recovered immediately after inoculation (0 hours incubation), use of a neutraliser, and the use of a smaller sized inoculum etc. Particularly for tier 2 and 3 testing, it is important that the chosen adaptations reflect the relevant conditions for which the claims must apply.

Specific tests which are recommended for certain uses are described under the sections for the different PTs.

5.5.4.1 Practical aspects for testing bacteria

A relevant study that proves the need for a biocide and its efficacy as a preservative against bacteria must have the following features:

a. The test must be performed in a range of relevant model matrixes that the claim of efficacy is made for (e.g. dishwasher liquid, paints, glues, textiles, etc);

b. The test has to be performed in relevant environmental conditions (temperature, type of matrix, humidity);

c. Control samples without the addition of a biocide must be included during the whole test. These control samples must be handled identically to the other samples, except that they must have no biocide included. The study must include replicate sub-samples for each treatment (minimum of 3; if less than 3 replicates, then explain and justify).

d. For preservative uses, the control samples should typically show growth (e.g. indicated by an increased number of cfu) during incubation and this has to be documented. If no growth in the control samples can be seen, this could indicate that only the dormant stages of bacterial cells, without active metabolism, are present in the matrix. The treated samples should show statistically significant effects as compared to the controls;

e. Only if growth cannot be proven by increase in cfu, data concerning other factors like e.g. CO_2-emission, O_2 depletion, change of pH, colour change or disintegration of the matrix should be used to demonstrate the need of preservation of a matrix by the active ingredient or preservative;

f. Relevant bacteria for the intended use have to be tested.

5.5.4.2 Practical aspects for testing fungi

A relevant study that proves the need of a biocide and its efficacy as a preservative against filamentous fungi is in many ways the same as for bacteria, but an attempt to count colony forming units of thread-like mycelia after incubation in liquid systems is bound to fail for several reasons:

- It is impossible to take a representative aliquot from the incubated test vessel since the mycelia tend to conglomerate into pellets of different sizes (often blocking the tip of a pipette).
- Different seized fragments of mycelium and spores that are dormant in the matrix form colonies on a petri dish and their origin cannot be differentiated and so their numbers do not reflect the increase in biomass that has occurred.

However, counting cfu is a practical option to measure the recovery rate of spores inoculated into liquids before spore germination (time 0 analysis) and for unicellular yeasts. At this stage, no mycelia have formed in the liquid, so no fragments will be counted as cfu and wrongly interpreted as growth. Therefore, after the control samples and the biocide-containing samples have been inoculated with spores, the recovery rate can be recorded by measuring colony forming units.

Ascomycetes and fungi imperfecti form thread-like hyphae and spores. Spores serve as dormant stages when environmental conditions are detrimental to growth. When growth conditions are favourable, the spores germinate and form a mycelium and maybe other spores. In liquids the fungal growth tends to form pellets. These can be very small or up to several millimetres in diameter. Furthermore, it is possible that a visible biofilm will accumulate at the sides of the test vessel, e.g. an Erlenmeyer flask or on the surface of the matrix. Both phenomena are visible by the naked eye and clearly demonstrate that the fungus has grown. In highly fluid materials this growth can be quantified by filtering the whole contents of the test vessel and then determining the amount of growth as dry weight. The use of replicates is an important factor in such tests. The number of replicates required by the methodology is not necessarily 3 which is the usual minimum; in such cases this needs to be explained and justified.

For testing solid materials, fungal growth is often assessed by optical appearance, using a rating scale from 0 (no growth) to 5 (>70% cover).

5.5.5 Testing conditions for specific states

5.5.5.1 Wet-state preservation and curative treatments

Preservation (PT 6, 13)
Challenge tests are generally employed for preservatives which must preserve liquid matrices, dispersions or fluids used in systems. The inoculum used and the strength of the inoculum depends on which claim must be supported. For preservation claims, growth needs to be shown in the untreated samples and prevention of growth in the treated samples. A larger population (generated by prior growth in an untreated matrix) may be more appropriate for demonstrating a curative effect. Some methods for wet-state preservation are compiled in Appendix 8, however, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor as to when and where these tests can be meaningful to prove /support a claim and when they aren't.

A series of concentrations of the active substance or the biocidal product should be employed in order to investigate which concentration achieves which level of efficacy. It is likely that the application rate in practice will vary depending on the in-use conditions of a biocidal product even though the matrix is identical, e.g. in a metal working fluid, where the in-use concentration is achieved by diluting the product at the point of use.

Curative Treatments (PT 6, 7, 11, 12, 13)
Suspension tests are generally employed for curative treatments of liquid matrices, dispersions or systems. A curative treatment might be applied to a system to reduce a population prior to employing a maintenance regime / treatment (e.g. PTs 11, 12 and 13) or it might be used prior to the addition of a preservative in either a final product, intermediate or a raw material (e.g. PT 6). A model matrix that has been inoculated with micro-organisms appropriate to the claim to achieve either growth or a stable population must be treated with the active substance / biocidal product and the effect measured after an appropriate contact time using a dilution plate count (methods described for wet state preservation can be employed to generate the model contaminated matrices / systems). The inoculum can comprise of aerobic or anaerobic bacteria, endospore forming bacteria, yeasts, fungal spores and / or mycelial growth as appropriate to the claim. A lg reduction relevant to the matrix and its use needs to be shown in the treated samples. Viability / growth should be shown to be maintained in the untreated samples. Replicate sub-samples must be employed (minimum of 3, but if the number of replicates required by the methodology is not 3 this needs to be explained and justified) and any differences that result should be shown to be statistically significant. Data from samples treated under field conditions can be used as supporting evidence provided that any effects shown can be attributed to the treatment applied.

5.5.5.2 Protection of solid material: PT 7, 9, 10

This section describes the nature and extent of data which should be made available to support the label claims for biocidal products within PT 7 through PT 10. The common denominator of these PTs is that they concern the treatment of solid material where use conditions can vary considerably, depending on the site and type of use of the material (e.g. treated wood to be used in constant contact with water compared to use in dry conditions; a film preservative to protect a bathroom sealant compared to protecting a house-façade). In contrast to liquid disinfectants or preservatives belonging to PTs 11, 12 and 13, where application often takes place on-site (that is where the target organisms occur), the treatment of materials can take place anywhere, for example where the material is manufactured or at a specific-treatment site. This may not necessarily be within the EU.

Use conditions are much more variable for these product types than they are for liquid disinfectants and liquid preservatives. Often, many different materials can be treated with the same biocide, and even more different articles can be manufactured from the treated materials, which are used in a wide variety of conditions. For instance, water absorption properties of different polymer materials vary and so does the release of the biocide. The concentration of the biocide has to be adapted accordingly. Biocides can be applied as a coating to fabrics or can be incorporated into the material by adding the biocide to the polymer before spinning or extrusion. This alters the fixation in or on the material and has an impact on performance. Materials and articles can be used indoors, outdoors, in wet, humid or dry conditions and at varying temperatures. All of this has an impact on performance. Simulating service life, as length, weathering conditions, temperature, leaching, laundering, etc. is crucial for testing of products within these PTs. Thus, efficacy testing for PT 7 through 10 requires a good description of the frame in which the biocide must perform. In many cases it will be impossible to test every material/substance combination; it might be feasible, however, to categorize different parameters: material, concentrations ranges, use (outdoor, indoor, temperature, humidity, use for load-bearing components, etc.) and to try to test representative, preferably worst-case, examples for every category. It is important though, to describe and justify which range the tested sample represents.

Model matrices

The array of possible material and biocide combinations is vast and phenomena observed in practice cannot always be reproduced in the laboratory. A model matrix has to be chosen which represents a certain type of material and which is relevant to the intended use. For example, plasticised PVC and polyurethane would be useful models for rigid or semi-rigid polymers and a room temperature vulcanised silicone would provide a useful model of a sealant etc. Relevance is the key factor. Thus, if a treatment is intended to protect natural fibres in service, then a natural fibre should be employed as the model. When more than one type of material (*e.g.* plastics, paints and synthetic fibres) can be protected by the biocide, then representative matrices that demonstrate the range of protection should be employed. Different materials can require different biocide concentrations due to varying release behaviour. It is also important to consider what the purpose of the end use is (*e.g.* in one application the biocide may provide essential protection of a matrix whereas in another it may increase durability). The objective is in any case to support the claims made.

Representative species

The species employed in any test should be relevant to the intended use (*i.e.* fungi should be employed if the material is affected by fungal growth, odour producing bacteria to be found on the skin should be employed for odour testing, etc.). Consortia rather than individual species should be employed (although mixing bacteria with fungi, algae *etc.* should, in general, be avoided, see 5.5.2). In exceptional cases, it can be acceptable to use individual species when justified, however, using consortia of micro-organisms can be a good option to reflect realistic use conditions but the use of individual species is also acceptable. The species employed in the tests should be relevant to the material under investigation especially where the prevention of the degradation of a material is intended. In many cases the organisms will be specified with the method. Very limited ranges of model organisms should be avoided where possible (*e.g.* the use of *A. brasiliensis* as the sole fungus). The test should include replicates (at least three) for both the treated and untreated variants.

Table 16: Examples

Claim	PT	Example Problem	Example Method
Fungicide is used to treat paint to prevent causing stains by mould growth in service	7	Painted panels exposed to weather become stained by mould growth and have to be re-painted more often.	BS 3900 Part G6 Painted panels inoculated with a mixture of spores of fungi known to colonise paints exposed to humid conditions for up to 12 weeks should show visual appearance of fungal growth. The treated sample should be free of it.
Fungicide is used to treat paper goods to prevent mould growth in service.	9	Labels used on wine and beer bottles become degraded and stained by fungi and difficult to read when stored in cellars and cool stores.	ASTM D 2020-03 Samples of untreated material should demonstrate a high susceptibility to fungal growth in the test. Treated samples should be free of growth.
Biocide with fungicidal and bactericidal properties is used to protect PVC sheet materials from spoilage and degradation in service	9	PVC sheet flooring used on solid floors can become colonised by bacteria and fungi on its under surface. This causes staining, cracking and detachment from the substrate.	ISO 846 Parts A and C. Samples of untreated material should support bacterial and fungal growth. Treated material should be free of growth.
Growth inhibition of moulds occurring on the plasters and walling in building structures	10	Surfaces of walls exposed to weather can be infected by saprophytic moulds.	Field trials: moulds growth should be shown on untreated material. Treated material should be free of moulds growth.

5.5.6 PT6 Preservatives for products during storage

In-can preservatives are included in many manufactured products, including paints, adhesives and binders. They are used to control micro-organisms that may be present in the product and which may cause deterioration prior to use. They therefore help to ensure product integrity during normal shelf life. Note: Food preservatives and cosmetics preservatives, which are used exclusively for this purpose, are not included in Product Type 6.

In order to grow in a manufactured product, a micro-organism must have access to both moisture (water) and a nutrient source. An extremely wide range of substances can act as a source of nutrition. These substances may be utilised by micro-organisms as they are, or following some form of conversion or degradation.

Utilisation of nutrition sources by micro-organisms results in the loss from the product of one or more components, leading to reduced integrity and spoilage. By-products of microbial growth also contribute to spoilage. Thus vulnerable products require an in-can preservative content for protection during the wet state, prior to use.

The broad group of wet-state preservatives for the purpose of storage prior to use has been divided into the sub-categories and sub-scenarios:

PT6.1 Washing and cleaning fluids and human hygienic products

6.1.1 Washing and cleaning fluids (human hygienic products)

6.1.2 Washing and cleaning fluids (general) and other detergents

PT6.2 Paints and Coatings (PN)

PT6.3 Fluids used in paper, textile and leather production (P)

6.3.1 Fluids used in paper production (Bulk raw materials in storage)

6.3.2 Fluids used in textile production (Bulk raw materials in storage)6.3.3 Fluids used in leather production (Bulk raw materials in storage)

PT6.4 Metal working fluid

6.4.1 Lubricants (P)

6.4.2 Machine oils (P)

PT6.5 Fuel

PT6.6. Glues and Adhesives

PT6.7 Mineral slurries and other matrices

Each of these sub-scenarios can be tested as described in 5.5.2 and 5.5.5.1. This can be summarised as follows.

- A relevant matrix must be chosen according to the intended use. This matrix should be selected in a way that it can easily support growth if no biocide is present. A reasonably high water content and organic matter (either from the matrix itself or added as a soiling agent) will allow for growth.
- If available, a standard that covers the matrix must be chosen (e.g. for glues you might choose ASTM standard D 4783). From this test protocol the test organisms, the method of cultivating the test organisms, duration of the incubation, incubation

temperature, etc. can be extracted and integrated into a test protocol that follows the principles outlined above (e.g. by reducing the size of the inoculum).

Examples for test protocols [24]that follow these principles are listed below. Other test methods which are commonly used for PT 6 can be found in Appendix 8. However, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor when and where these tests can be meaningful to prove a claim and when they aren't:

i. A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Polymer Dispersions, IBRG PDG 16-001;

ii. A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Aqueous-Based Paints, (IBRG2 P 16-001;

iii. Tier 1 Basic Efficacy Method for Biocidal Active Substances used to Preserve Aqueous-Based Products, (IBRG2, IBRG PDG 16-007.

These documents describe methods for determining the basic efficacy of biocidal active substances in an aqueous based matrix and are intended for the generation of tier 1 data. The impact of additional factors like temperature and chemical stability etc., depending on the claim, would need to be tested.

When a claim of an active is to reduce bacterial growth, all 3 methods work according to the same principles, but differ in the bacteria used as they are specific to the matrix and the strength of the inoculum (also refer to 5.5.4.1). When the active substance also claims to reduce fungal growth, it will be necessary to differentiate between unicellular yeasts and filamentous fungi as yeasts can be counted as colony forming units, whereas filamentous fungi cannot (also refer to 5.5.4.2).

The filamentous fungus *Geotrichum candidum* is an organism that forms filamentous chains of fragmented cells. These are special in so far as they disintegrate easily into single arthrospores. Enumeration of growth of this fungus can therefore be performed in the same way as for unicellular yeasts. Details for culturing this fungus are given in method ii (Paints).

Whereas methods i) deal with polymer dispersions and ii) deal with paints, the efficacy of preservatives in all other matrices in PT 6 are at this point tested according to a generic method shown under iii) above. It provides a unified approach and is for use with those materials that do not (yet) have a specific method available (e.g. surfactants, cleaning products, mineral slurries etc.). It is designed to satisfy the basic requirements described in this document. As with the above tests, it is based on a challenge test (multiple inoculations at weekly intervals) and has the same basic requirements.

5.5.7 PT 7 Film preservatives and PT 9 Fibre, rubber and polymerised materials preservatives

Uses within PT 7 (film preservatives) and PT 9 (fiber, leather, rubber and polymerized material preservatives) often overlap. Sometimes, PT 7 and 9 differ only in the manner of application: the biocide can be applied as a coating layer onto the material or it can be incorporated into the material. Thus, the described requirements and principles apply in the same way to both PTs.

[24] IBRG website for test protocols: http://ibrg.org/Methods.aspx

When selecting the appropriate method, consideration must be given to the release mode characteristics of a particular biocide/material combination. Some biocides have a very low solubility in water and hence are emitted at a very low rate from a matrix. This may be sufficient to protect a material that is inherently highly susceptible and which micro-organisms may penetrate and colonise. However, if a test (*e.g.* ISO 16869) relies on the emission of the biocide from the matrix into an agar layer to measure the effect, the test would indicate that such a biocide has no function. Other materials, which are damaged by growth on their surface (especially where soiling is present) due to the production of extracellular enzymes, may fail to be protected by a biocide with such a low emission rate. Thus, the choice of method will be highly dependent on the characteristics of the material as well as the biocide. The applicant should justify this for the product under evaluation.

5.5.7.1 Simulation Tests (tier 1 testing)

The ideal test method would present a material to a consortium of relevant test organisms under conditions that simulate real life realistically. This would produce effects that are identical to those observed in practice and allow a treatment to be identified with precision. There are methods that come closer to this ideal than others. For example, BS 3900 Part G6 (Appendix 6) exposes painted panels that have been inoculated with a mixture of spores of fungi known to colonise paints to humid conditions, free of external nutrients (although these can be added with the inoculum if necessary) for up to 12 weeks (see Figure 8). The resulting growth on untreated coatings has a visual appearance very similar to that observed in practice. For tier 2 pre-exposure, leaching or artificial weathering can be used to help explore service life. A comparison can be made between treated and untreated variants of a formulation. A similar test, that forms the basis of many of the military standards and specifications, is BS EN 60068-2-10:2005 (see Appendix 6); this test is applicable to a wider range of materials. Again, samples are inoculated and incubated under conditions intended to simulate real life or at least be optimal for fungal growth.

Figure 8: Example of a Simulated Growth Test

BS 3900 Part G6, Method Overview:

Replicate sub-samples of both treated and untreated variants of each coating are sprayed with a suspension of spores of a range of fungi known to colonise surface coatings. The samples are then transferred to a humid chamber and incubated for up to 12 weeks. The extent of growth is assessed using a rating scale and this, as well as photographs of the panels, are presented as the results.

Rating scale: 0 = no growth, 1 = trace to 1% cover, 2 = 1 - 10% cover, 3 = 10 - 30% cover, 4 = 30 - 70% cover and 5 = > 70% cover

There is no pass/fail criterion in the standard but many workers in the coatings industry consider that growth represented by a rating of 2 is the maximum that would normally be tolerated. An example of growth on an untreated coating is shown on the left.

Example for growth level 5.

Modifications of these methods have been made to allow them to study the effects on algae (the IBRG algal test method for surface coatings) and, less commonly, bacteria. Effectiveness is assessed in these tests by visual appearance, measuring loss of weight or determining changes in the physical properties of the material (*e.g.* resistance to bending or extension under load). As with all biological tests, some degree of replication will be essential and tests should employ, as a minimum, three replicate sub-samples of each variant. Simulation tests are indeed very useful and provide valuable information especially for specific material/biocide combinations and can be correlated in some cases to service expectations. However, they can take a long time to perform and, in many cases, need to be adapted in some manner to accommodate a specific material.

5.5.7.2 Tests based on artificial growth media (tier 1 testing)

By far the most commonly used methods for studying the performance of biocides intended to protect materials are those based on artificial growth media such as agar plates. For example, both ISO 846: 1997 and ASTM G21-09 are used widely in the plastics industry to measure the performance of fungicides in formulations (also ISO 16869: 2008). ISO 846 allows for studies into the susceptibility of plastic formulations to fungal and bacterial deterioration by attempting to make the plastic the sole source of nutrients for the organisms used, as well as providing a variant that provides an external source. It also includes a service life simulation test variant in which samples are buried in soil and then examined for loss of weight and strength (extremely useful in industries manufacturing pipes and cables). Although making the plastic the sole source of nutrients might seem like the ideal way to examine the ability of a biocide to protect the material, in many instances it is the presence of soiling that leads to colonisation and subsequent damage to the polymer (sometimes referred to as bio-corrosion). Thus, for certain polymers, the presence of

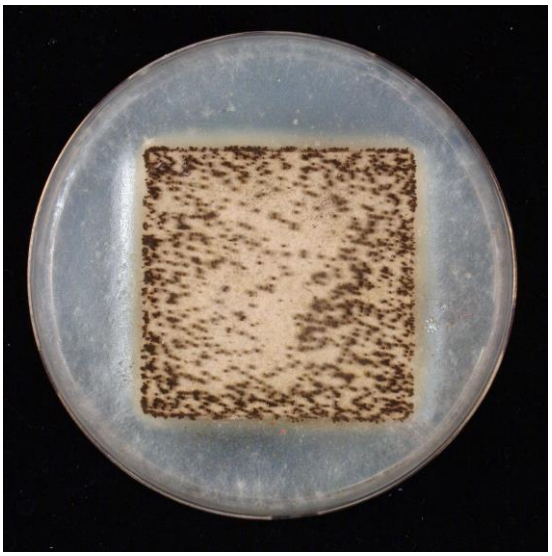
external nutrients is essential in determining the efficacy of a biocide. In many instances a consortium of organisms is required to effect colonisation and deterioration of the material and, in general, methods that employ consortia should be selected.

Similar testing technologies as those used for plastics exist for certain textiles, paper and surface coatings. The most commonly used are listed in Appendix 9; However, please note that these test methods are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor when and where these tests can be meaningful to prove a claim and when they aren't. A description of the basic principles of tests on artificial growth media is given in Figure 9 using ASTM G21 as an example.

The huge disadvantage of agar-plate based tests is the interference of the growth medium with the biocide. The biocide can diffuse into the agar, demonstrating an effect there but at the same time be diluted in the original matrix. A less soluble substance, which does not diffuse into the agar, may in contrast show a false negative effect. For these reasons, a simulation test is always to be preferred over an agar-plate based test.

Figure 9: An Example of an Agar Plate Based Test

ASTM G21, Method Outline:



Replicate samples of both treated and untreated material are embedded in a mineral salts-based agar medium. The sample and surrounding agar are then inoculated with the spores of a mixture of fungal species known to colonise plastics. The plates are then placed into chambers in which the humidity is maintained at > 85% RH for up to 28 days. The samples are then inspected for the presence of fungal growth. Typical growth on an untreated material is shown in the plate on the left.

Growth on Untreated Plastic

5.5.7.3 Tier 2 Testing

Depending on the intended use, pre-exposure, leaching or artificial weathering can be used to help explore service life. The relevance of the chosen parameters should be explained. There are no special tests or designs available for tier 2 testing. Basically, the same methods as in tier 1 can be applied except that the tested material undergoes pre-treatment. In some cases, ageing norms can be employed (e.g. adaptations of EN 73:2014[25], EN 84:1997[26], which are both developed for treated wood). In other cases, variations of the tier 1 methods can be used (as for example the soil burial variant of ISO 846 as described above). It is

[25] Accelerated ageing test of treated wood prior to biological testing. Evaporative ageing procedure
[26] Accelerated ageing tests of treated wood prior to biological testing. Leaching procedure

particularly important to show growth or damage on the untreated material under service-life conditions.

In some cases it may not be necessary to use an artificial inoculum for tier 2 tests. It may be possible to use a test medium colonised naturally so that it is representative of the organisms that are typically encountered during the use of the product. It may be valid to use lower levels of contamination such as those encountered in practice. In some cases there may be a need to include application-related test-organisms in addition to standard test-organisms. In any case, the applicant should provide a rationale as to why the test organisms are relevant for the respective application/s of the preservative. Representatives for all claimed organisms should be tested.

5.5.7.4 Tier 3 Testing

In some cases, tier 3 testing might be needed to support specific claims. These can be field trials where treated materials are compared to untreated materials in use. For example, treated house facades could be compared to untreated house facades in the same area and the time until re-painting is needed could be measured. Likewise, the replacement time for untreated buried cables compared to treated ones can be studied in a field trial. Care has to be taken that the conditions for the treated and untreated materials are the same or at least comparable and that other parameters than the parameters observed are not influencing the results. The validity of the conclusions may need to be reinforced by statistical analysis etc., especially if any differences observed are small.

Table 17: Basic Requirements for a Valid Test Protection

The following summary provides a guide to the basic requirements for a valid test:

i. A relevant model matrix should be chosen to represent the material(s) which must be protected;
ii. Relevant use conditions should be chosen in terms of humidity temperature and soiling;
iii. An untreated variant of the test material must be included and show the pattern of growth/deterioration that the biocide is intended to prevent at the end of the test;
iv. The test should employ organisms that are relevant to the material/problem being addressed;
v. Tests that employ a consortium of organisms should be favoured over those that use single species;
vi. A minimum of three replicate test pieces of both treated and untreated materials should be employed;
vii. The final data should include either some indication of the impact of service conditions on the performance of the treated material/article or data from an ageing.

5.5.7.5 Prevention of Odour by odour-producing micro-organisms

With most of the biocidal functions within PT 7 and 9, test conditions simulate in-use conditions rather well and the effects of microbial growth or activity can be observed quite easily. With the control of odour, this is much harder to achieve in a laboratory test, as odour often cannot be measured in a simple manner.

Laboratory tests to simulate odour production are currently not available, though some work is done to develop such tests (for example a test to inhibit the bioconversion of L-leucine to iso-valeric acid, representing a dominant compound of foot-odour). Thus, at present, the prevention of odour is in most cases measured indirectly by measuring microbial inhibition.

There are two major types of test that have traditionally been used with textiles (and related materials). The first major group employs agar plates and the other major group uses suspension in an aqueous medium. In both cases, the impact of a treated textile on populations of (usually) bacteria are studied. An overview is given in Appendix 10; however please note that the test methods listed are not necessarily appropriate to use; they are listed with comments to give an orientation for the assessor when and where these tests can be meaningful to prove a claim and when they aren't.

Agar plate-based tests

Agar plate-based tests are not recommended. These tests have almost no useful utility in measuring effects intended to control odour in textiles. Such tests rely on the biocide migrating from the textile into the agar medium at sufficient concentration to inhibit the growth of bacteria either seeded into the agar or placed onto it (see Figure 9). The diffusion characteristics vary hugely from one biocide to another and from one textile to another and the growth medium itself presents a large soiling load to be overcome by the biocide. Larger areas clear of growth are often associated with more potent effects but they could be attributed equally to differences in the leaching rate of a biocide from a material.

Suspension tests

The second major group, the suspension tests, measure changes in the size of a population following contact with a treated textile. A number of protocols are described in Appendix 10. However, most employ relatively high concentrations of nutrients in the suspending medium so that their application, like the agar diffusion methods, can lead to over-treatment of textiles. Thus, these methods should not be used. By using lower concentrations of nutrients in the suspending medium and using pre-treatments such as laundering, these methods can be adapted for use in measuring effects on odour. Such an adaptation has been applied in the OECD tier 1 method for treated articles (porous materials[27]) and the IBRG Textile Method[28]. These are described schematically in Figure 10 and are based on the 'germ' count or absorption phase of ISO 20743: 2007 where the amount of nutrients present in the cell suspension has been reduced substantially.

Many treated materials would certainly be capable of demonstrating activity in a suspension test. Activity against a consortium of bacteria (e.g. against a range of Gram Positive and Gram Negative bacterial species such as *Staphylococcus epidermidis*, *Corynebacterium xerosis*, *Proteus vulgaris, Escherichia coli, etc.*) would probably inhibit the production of odour. However, excess exposure of the skin of the wearer should be minimized as far as possible. Therefore, tests adapted to textile treatments such as the OECD tier 1 method and the IBRG Textile method (Figure 10) are preferable.

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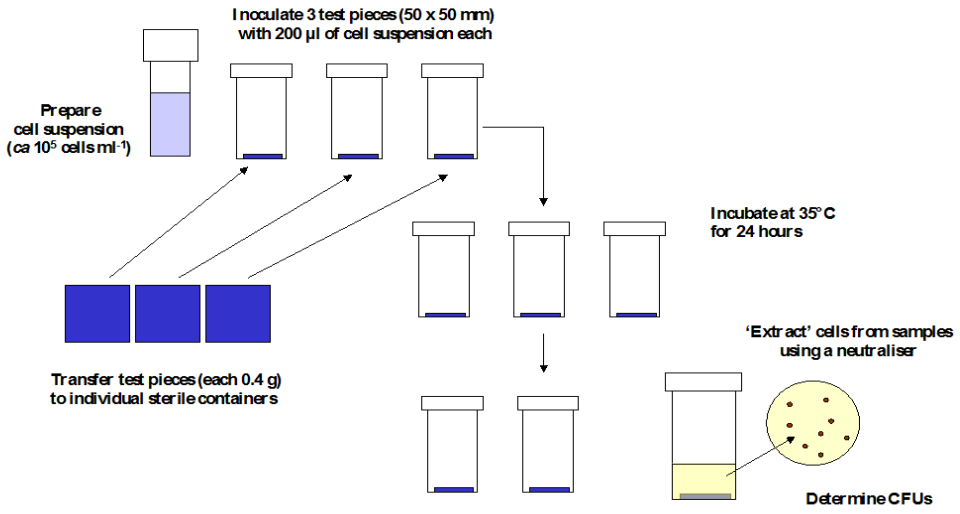
[27] OECD (OECD ENV/JM/MONO(2014)18: Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials (OECD Series on Testing and Assessment No. 202 and Series on Biocides No. 8).

[28] IBRG, International Biodeterioration Research Group (2013): Quantitative Method for Evaluating Bactericidal Activity of Textiles and Porous Materials and Articles. IBRG TEX/13/005 (www.ibrg.org).

Figure 10: OECD/IBRG Tier 1 Textile Test



Tier 2 testing

In many cases, a large fraction of the active substance incorporated in a textile is lost during laundering, either through emission of loosely or only partially bound material or associated with loss of fibres (lint). This also means that there is potential for active substances to be transferred from treated materials to non-treated materials when laundered together. In general, the emission rate is rarely continuous either to the environment or to the wearer. Moreover, other chemicals from the textile treatment as well as chemicals used in the laundering process might interfere with the function of the biocide.

In general, the effects required to prevent the formation of odour in shoes and apparel are subtle. The greatest demand on them is usually in maintaining activity following multiple laundering cycles. Therefore, simulation of service life conditions by laundering and ageing are essential. Care must be taken to maintain the functionality and to minimise excess exposure of the environment through emissions of the biocide in use, during cleaning and at the time of disposal. The method described in Figure 10 (as well as chemical analysis) in combination with laundering cycles can be useful in measuring the maintenance of efficacy in service.

An active substance or a biocidal product is often intended to treat a wide range and mix of textile types with a wide variety of anticipated demands and expectations of durability. It might be difficult to address every potential combination and garment type. However, studies on typical textile blends could be used to provide appropriate efficacy. Some examples are given in Table 18 below.

Tier 3 testing

At present the only truly reliable methods for demonstrating anti-odour functionality is through replicated and statistically designed wearing trials. Tier 1 and 2 tests described above can provide useful data related to durability *etc.* but care must be taken when interpreting the data they produce. For example, a treatment may be applied to only certain parts of a garment or shoe or it may be present on only a certain number of filaments in the weave of a textile. In the bioassay, the inoculum is dispersed throughout the whole of the sub-sample of textile and any active substance released would be able to migrate throughout that inoculum whereas in use, this may not occur. The humidity produced by bodily excretions might trigger less release of the biocide than the liquid suspension the textile is covered with in the test. The bacterial populations present on the skin might be less affected by the biocide as compared to the testing consortium employed. Consequently, user trials are proposed as reliable methods to prove anti-odour effects, especially in case of textiles, but also suitable microbiological studies with relevant odour-causing micro-organisms can be acceptable ways to prove anti-odour claims. A standard with human assessors which could possibly be adapted to test anti-odour claims is EN 13725.

Table 18: Odour: Example Claims, Problems and Testing Approaches

Claim	PT	Proof Required	Example Method
Carpet is treated to prevent odours caused by mould growth.	9	Data should show that the treated carpet does not support fungal growth whereas the untreated one does. The effect should be shown to be sufficiently durable.	A method such as AATCC 174 can be used to demonstrate resistance to fungal growth. For active substances that do not migrate from the fibres/backing a cabinet-based simulation test may be more appropriate. Activity should be shown to persist following simulated ageing.
A sports vest is treated to inhibit the production of odour.	9	Data from a field trial should show that odour is reduced in treated sports shirts when compared with untreated ones. The effect should be shown to be of sufficient durability during service life to match any claim made.	Wearing trial or scientifically valid odour based simulation study. A comparison of the effectiveness both before and after simulated ageing/washing should be performed. This could be performed either through field trials, simulation tests or the use of a test such as the OECD tier 1 method. The latter could be used to demonstrate that sufficient activity is still present after washing/ageing to elicit an antimicrobial effect.

5.5.8 PT8 Wood preservatives

General Introduction

This document deals with the evaluation methodology of efficacy tests for wood preservatives biocidal products that are applicable in the frame of the EU Biocidal Products Regulations (BPR) for the authorisation of biocidal products (BPR Annex VI).

The document is not intended to replace standards, standardized methods or other methods used as reference for developing the required data. It is considered as scientific guidance and the reader is advised to refer to the standards themselves or appropriate literature in case details should require further clarification.

The aim of this document is to provide a common base for the assessment of the efficacy for the biocidal product authorization for PT8 products for the applicants and the Competent Authorities (CAs).

Although alternative test methods could be taken into account, this document is mainly based on the EN 599-1 standard for preventive uses and on the EN 14128 standard for curative uses.

This document covers the products used for the preventive treatments of wood (including the saw-mill stage), by the control of wood-destroying or wood-disfiguring organisms (temporary treatments of logs in the sawmill or log yards, temporary treatments of green sawn timber, treatments of sawn timber including round timber, treatments of wood based panel) and products used for the curative treatments of sawn timber in service.

For product already on the market before entering into force of the standards (in 1990 for EN 599 and in 2004 for EN 14128):

- Efficacy data on the product should be provided.
- The assessment of the product efficacy should be based on expert judgement;
- Some data taken from the literature or used in certification could be accepted on case by case basis.

When the data are not enough robust to demonstrate the efficacy of the product, new tests according to EN 599 and/or EN 14128 will be required.

At the review time of this document, it has been chosen to include the catalogue of uses in the Chapter 7 of the Technical notes for guidance (TNsG) on product evaluation (PT8). The inclusion of the catalogue of uses to this document is to provide a common basis to harmonize the claims of the product. It will facilitate in a second time the mutual recognition by listing the elements of the claim in the same order and using the same terminology. On the label, the categories related to the product should be presented as described in the following paragraphs. The codes increase the readability of this document and are not expected on the label.

Concerning the updating of this document, it should be considered as a living document and will be reviewed on a regular basis and updated if necessary, under ECHA’s procedures.

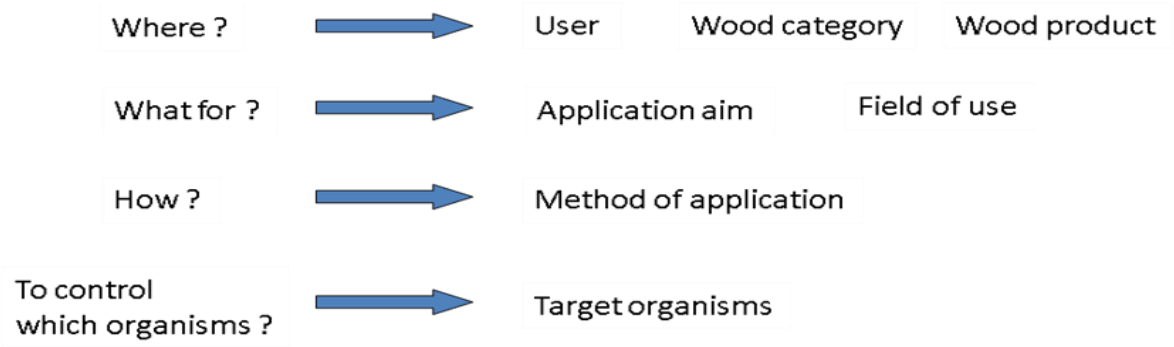
The tests should be performed according to the current version in force of this document. Any tests initiated before the endorsement of the new version remain acceptable.

5.5.8.1 Label claims

In order to harmonize the efficacy issues, it is proposed that the different uses of the product are presented following the proposal below. This should follow the order of the categories listed below.

The aim of this categorisation is to have an explicit answer on the following questions:

- Where is the product used?
- What is the product used for?
- How is the product used? To control which organisms?



The data which support the efficacy should also follow this format.

The main categories that should be present on the label are listed in Table 19 and are detailed in the following paragraphs.

Table 19: Different categories and the related product codes

Categories	Code for product
User category	A.xx
Wood category	B.xx
Wood product	C.xx
Application aim & Field of use	D.xx & E.xx
Method of application and rate	F.xx
Target organisms	G.xx

5.5.8.1.1 User Category (Code for Product A.xx)

Information on the intended users of the product has to be presented on the label, the different user categories are presented in Table 20.

Table 20: User categories

User Category	Example	Product Code
Non-professional/general public	Product used at home by consumers	A.10
Industrial	Industrial applicator	A.20
Professional	Pest control operator	A.30

5.5.8.1.2 Wood Category (Code for product B.xx)

This section deals with the wood category and not the use classes as defined in EN 335 standard. From an efficacy point of view, in EN 599-1, annex D the wood timbers are divided into two categories: softwood and hardwood.

Softwood and hardwood species of timber react differently to the degree and the type of attack by certain biological agents.

In most cases, the tests are performed with softwood. In some cases it is acceptable for this data to be read-across to hardwoods, but in other cases specific testing against hardwoods is required. (see EN 599-1).

Table 21: Wood categories

Wood Category	Product Code
Softwood	B.10
Hardwood	B.20

5.5.8.1.3 Wood Product (Code for product C.xx)

Table 22 below describes the types of wood products that are used as building materials or in the manufacture of furniture. Wood products are divided in two main categories: solid wood and wood based panels. Based on European standards, wood based panels are divided in four categories: plywood (EN 636), OSB (EN 300), Particles (EN 309 & EN 312) and Fibers (EN 622).

Table 22: Wood product categories

Wood Category	Product Code
Solid wood	C.10
Reconstituted solid wood *Engineered solid wood products produced by processes involving pressure, adhesives and binders*	C.11
Panels	C.20
Plywood panels	C.21
OSB panels	C.22
Particles panels	C.23
Fibers panels	C.24

5.5.8.1.4 Application aim and field of use

5.5.8.1.4.1 Application aim (code for product D.xx)

A preventive treatment is used to prevent sound wood from being infected by wood destroying agents and/or disfiguring fungi. The curative treatment is used to kill infective organisms that have already attacked the wood, to prevent them from spreading in the rest of the wood.

The preventive treatments are most of the time used during the manufacturing process but can also be done when the wood is in its service situation (e.g. framework of the building, a bridge.).

According to the fact that a product can be used in wood preventive treatments, in curative treatments and sometimes both, and according to the fact that wood preservative and curative treatments are not covered by the same treatments, it is proposed to split the application aims as presented in Table 23.

The aim of this classification is to ensure having the same classification throughout the EU.

Table 23: Application aim

Application Aim	Kind of Treatment	Product Code
Preventive	Temporary preventive treatment / logs	D.10
	Temporary preventive treatment / green sawn timber	D.20
	Preventive treatment / blue stain in service	D.30
	Preventive treatment-use class (cf. the following section for the field of use – code E)	D.40
Curative	Curative treatment / wood in service	D.50
Preventive	Other (for e.g. pole maintenance)	D.60

5.5.8.1.4.2 Field Of uses (Code For Product E.xx)

The use classes described in EN 335:2013 are defined in terms of service conditions, with reference to the generalised moisture content and the prevailing biological agents of deterioration. The different classes (and their related application codes) are presented in Table 24.

- Use class 1: situation in which the wood or wood based product is inside a construction, not exposed to the weather and wetting;
- Use class 2: situation in which the wood or wood-based product is under cover and not exposed to the weather (particularly rain and driven rain) but where occasional, but not persistent, wetting can occur;
- Use class 3: situation in which the wood or wood-based product is above ground and exposed to the weather (particularly rain);
- Use class 4: situation in which the wood or wood-based product is in direct contact with ground or fresh water;
- Use class 5: situation in which the wood or wood based product is permanently or regularly submerged in salt water (i.e. sea water and brackish water).

Use class 3 is split into two sub-classes:

- 3.1: wood and wood based products will not remain wet for long periods. Water will not accumulate;
- 3.2: wood and wood-based products will remain wet for long periods. Water may accumulate.

The use classes 4.1 and 4.2 described in the former version of the EN 335 standard (2009) have been merged into a single use class 4, including both wood in exterior, in ground and/or freshwater contact.

Table 24: Different fields of uses

Field of Uses	Product Code
Use class 1	E.10
Use class 2	E.20
Use class 3*	E30
Use class 3.1	E.31
Use class 3.2	E.32
Use class 4	E.40
Use class 5	E.50

* includes use class 3.1 and use class 3.2

5.5.8.1.5 Method of application and application rate (Code for product F.xx):

The various methods available can be broadly split into three groups:

- **Superficial treatments:** Such non-pressure processes include brush, spray, roller, pad application and immersion (dipping) processes (where the wood can be in contact for preservative for periods of time ranging from a few minutes to several hours). The application rates are commonly expressed in g/m^2, ml/m^2.
- **Penetrating treatments:** Such processes include the vacuum pressure, alternating oscillating pressure, double vacuum and non-pressure processes such as diffusion treatments. The application rates are commonly expressed in kg/m^3.
- **Other treatment methods:** For application methods different from those described above (fumigation, injection), either specifically relevant data or some justification for non-inclusion of data (i.e. details on penetrability/retention, etc.) will need to be provided to the CA for consideration.

Some PT 8 products are designed to be used with a top coat, e.g. primers for window framing. If a top coat is needed according to the manufacturer, this must be applied with the product. When a more general use is envisaged, generic coating materials can be used according to the norms performed.

Table 25: Method of application

Method of application	Product Code
Superficial application / brush/roller/pad treatment	F.10
Superficial application / spray treatment	F.11
Superficial application / flow coat /aspersion	F.12
Superficial application / foam treatment	F.13
Superficial application / dipping treatment	F.14

Method of application	Product Code
Injection	F.20
Pressure process	F.30
Pressure process / vacuum pressure impregnation	F.31
Pressure process / double vacuum	F.32
Fumigation	F.40
Fumigation bubble	F.41
Pole in services fumigation	F.42
Mixing with glue and mortar	F.50
Diffusion	F.60
Solid pellets / rods	F.61
Pole bandage / wrapping / pad application	F.62
Other application methods	F.70

5.5.8.1.6 Target organisms (Code for product G.xx)

This section describes the main categories of target organisms, in relation to the claimed uses of the product, either for treatments to prevent biological attack, or for curative treatments to disinfest or to eradicate existing attack.

Appendix 11 gives more information on the principal target organisms.

There are a number of possible effects on target organisms resulting from the proposed use of a wood preservative product. The efficacy data for a wood preservative must be suitable to demonstrate the efficacy of products applied as either pre-treatments to prevent biological attack, or as curative treatments to disinfest or to eradicate existing attack. These may be in a variety of forms; they may yield toxic values, mortality values, subjectively derived ratings or effective retention values.

On the claimed matrix, the target organisms against which an efficacy is claimed must be clearly described. For the purpose of harmonisation, it is proposed that the target organism presented in Table 26 should be used, although these should not be considered as an exhaustive list. The species presented below are the species being representative of wood attacking organisms. For specific claims, efficacy data against each named target pest will be required.

Table 26: Examples of target organisms for wood preservatives

(N.B. these examples are not intended to be exhaustive with respect to target organisms or prescriptive with respect to data to be generated).

Target organisms				
Common English term	**Code F for product**	**Target organisms according to EN 1001**	**Classification**	**Scientific name**
Fungi			**Fungi**	
Wood rotting fungi				
Wood rotting basidiomycetes	G.10	Brown rot fungi	Basidiomycetes	e.g. *Gloeophyllum trabeum*
	G.11	White rot fungi	Basidiomycetes	e.g. *Coriolus versicolor*
Soft rot fungi	G.12	Soft rot fungi	Ascomycetes, Deuteromycetes	e.g. *Chaetomium globosum*
Wood discolouring fungi	G.21.1	Sapstain fungi (bluestain mainly)	Ascomycetes, Deuteromycetes	e.g. *Ophiostoma piliferum (Ceratocystis pilifera)*
	G.21.2	Bluestain in service	Ascomycetes, Deuteromytcetes	e.g. *Aureobasidium pullulans*
	G.22	Mould fungi	Ascomycetes, Deuteromycetes,	e.g. *Aspergillus niger*
Insects			**Insecta**	
Beetles	G.30	Wood boring beetles	Coleoptera	
	G.31	House longhorn beetle		e.g. *Hylotrupes bajulus*
	G.32	Common furniture beetle		e.g. *Anobium punctatum*
	G.33	Powder post beetles		e.g. *Lyctus brunneus*
	G.40	Fresh wood insect	Coleoptera	e.g. *Scolytus* spp.
Termites	G.50	Termites (genus claimed)	Isoptera	
	G.51	Subterranean termites (genus claimed)		e.g. *Reticulitermes* spp., *Coptotermes* spp.
	G.52	Drywood termites (genus claimed)		e.g. *Cryptotermes* spp.
	G.53	Tree termites (genus claimed)		e.g. *Nasutitermes* spp.
Wood destroying marine organisms	G.60	Marine borers (genus claimed)		
	G.61	Mussels	*Teneridae, Pholadidae*	e.g. *Toredo* sp., *Martesia* sp.
	G.62	Crustaceans	*Isopoda, Amphipoda*	e.g. *Limnoria* spp., *Chelura* spp.

5.5.8.1.7 Examples of a claimed matrix

To illustrate the previous sections described, the following table gives an example of claimed matrix based on the categories from the catalogue of uses. This framework should be followed for the efficacy claim's part of the label. Only the categories and the matrix wordings (not the code) are expected to be listed on the label.

This matrix allows a harmonisation of the efficacy elements presented in the dossier for product authorization. Elements in the claimed matrix must be present on the physical label.

Table 27: Examples of claim matrix based on the application codes for product

Categories	Matrix Wording	Code for Product
Label 1		
User category	Industrial	A.20
Wood category	softwood and hardwood	B.10; B.20
Wood product	solid wood	C.10
Application aim and field of use	preventive treatment - use class 3.2	D.40; E.32
Method of application and rate	superficial application/dipping treatment application rate: 100 g/m² in the analytical zone a top coat must be applied. pressure process/vacuum impregnation application rate: 50 kg/m^3 in the analytical zone	F.14 F.31
Target organisms	wood boring beetles	G.30
	termites (genus *Reticulitermes*)	G40
	brown rot fungi	G.10
	white rot fungi	G.11
Label 2		
User category	Industrial	A.20
Wood category	softwood and hardwood	B.10; B.20.
Wood product	solid wood	C.10
Application aim and field of use	preventive treatment - use classes 2, 3 and 4	D.40 - E.20; E.30; E.40
Method of applicationand rate	superficial application/dipping treatment application rate in the analytical zone: UC 2: 80 - 120 g/m² UC3 (coated): 100 – 160 g/m^2 pressure process/vacuum pressure impregnation	F.14 F.31

	application rate in the analytical zone: UC2: 30 kg/m^3 UC3: 40 - 70 kg/m^3 UC4 (softwood): 80 – 150 kg/m^3 UC4 (hardwood): 100 – 150 kg/m^3	
Target organisms	brown rot fungi	G.10
	white rot fungi	G.11
	soft rot fungi	G.12
	wood boring beetles	G.30
	termites (genus *Reticulitermes*)	G.40
Label 3		
User category	Industrial	A.20
Wood category	Softwood	B.10.
Wood product	solid wood	C.10
Application aim and field of use	temporary preventive treatment - use class 1	D.20 E.10
Method of application and rate	superficial application / dipping treatment application rate 100 g/m² in the analytical zone	F.14
Target organisms	Sapstain	G.21.1
	mould fungi	G.22

5.5.8.2 Available data

5.5.8.2.1 Standard test methods

When considering the overall evaluation of proposed claims, CAs should ensure that the test methods (data, method of application and application/dose rates used in the tests, product tested) are appropriate to demonstrate the efficacy claimed on the label for the product.

Many standard protocols currently exist to test wood preservatives; the lists of standards for the efficacy assessment of wood preservatives are available on the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy]. For PT8, the CEN standards are highly recommended.

Two main categories of treatment are described:

- Preventive treatments, which are covered by EN 599-1;
- Curative treatments, which are covered by EN 14128.

Some other treatments (C.20: green sawn timber) are covered by other standards (e.g. CEN TS 15082).

It is highly recommended to perform the studies according to these standards. If the standards are not applicable or suitable, the applicant may adapt the methodology or use

another method (including his own method). When a standard is modified or when a non-CEN standard is used, a robust justification and description have to be provided. For very specific cases, tests or ageing procedures could be waived with a robust justification. The study submitted has to provide a clear answer to the issue.

In the general part of the TNsG on data requirements it is mentioned that the test (and the data generated) should be based on sound scientific principles and practices. Compliance with quality standards is highly recommended.

In the TNsG on product evaluation, it is mentioned that for efficacy testing, the principles of Good Laboratory Practice (GLP) are not required by the legislation. However this guidance indicates that the spirit of such principles should be applied for the testing of efficacy.

Particular attention should be paid to:

- what information is needed to substantiate a 'claim matrix';
- the Quality Assurance procedures which should be adopted (cf. ISO 17025 for testing and certification);
- the overall evaluation of the data package when the completeness and adequacy of the data are compared with the label claim.

For products intended for application as solids, pastes or encapsulated forms and those intended for curative (in-situ) use, modification of the relevant protocols/testing strategies may be done or other direct evidence may be submitted on their potential efficacy against the claimed target organisms (e.g. for pastes such evidence could be in the form of penetrability and retention characteristics).

The test methods used to provide data should be relevant to the target organisms and application processes claimed on the label (see EN 599-1 and individual test standards).

It has to be noted that in some cases, a different formulation from which an authorization is sought could be tested. The results could be accepted by the RMS in a case by case approach (see section 5.5.8.3 of this guidance and Annex A of the EN 599-1 and EN 14128). A full composition of the tested product and a robust justification why the test is relevant should be provided.

For EN113, where the protocol states that several organisms have to be tested in order to fulfil the efficacy criteria, it is recommended that all testing is done in the same laboratory at the same time. The sponsor must have the right to provide his rational for justification why the simultaneous testing may have not been followed. Derogation (inter alia) is acceptable i.e. in the following cases:

- where the test was performed with limited organisms and later completed with additional organisms which could be tested in another laboratory (extension of claim);
- where the laboratory cannot run the test with specific targets;
- where the laboratory has ceased to provide services;
- in the case where a 'simultaneous test' is not available, but valid tests (according to the criteria in the standard) are available.

Table 26 and Table 28 are informative for the test methods used. The user should also refer to EN 599-1 or EN 14128 depending on the claims.

Table 28: Preventive treatments: List of available standards and other methods used in wood preservation

Organisms	Code for product	Temporary treatment of logs	Temporary treatment	Treatment of solid wood (List of standards mentioned in the tables 1 to 5 of EN 599-1) Note 1: In some conditions, ageing tests (EN 84, EN 73) or natural weathering are required (see EN 599-1) Note 2: It is highly recommended to refer to EN 599-1 to determine the tests to be done in accordance with table 1 to 5 of EN 599-1					Treatment of wood based panels[29]
				Use Class 1	Use Class 2	Use Class 3	Use Class 4	Use Class 5	
Brown rot fungi	G.10				EN 113	EN 113 EN 839 EN 330	EN 113 EN 252	EN113	ENV 12038
White rot fungi	G.11					EN 113 EN 839 EN 330	EN 113 EN 252	EN113	ENV 12038
Soft rot fungi	G.12						ENV 807 EN 252	ENV 807	
Sapstain fungi	G.21.1	No CEN standard*	No CEN standard*						
Bluestain fungi	G.21.2		No CEN standard*		EN 152	EN 152	EN 152	EN 152	
Mould fungi	G.22		No CEN standard*			No CEN standard			
Wood boring beetles	G.30			EN 46 EN 47 EN 49-1 EN 49-2	EN 46 EN 47 EN 49-1 EN 49-2	EN 46 EN 47 EN 49-1 EN 49-2	EN 47 EN 49-2 EN 20-2	EN 47 EN 49-2 EN 20-2	

[29] For wood based panels, the reader is aware that standards can be adapted in specific cases (e.g. CEN/TS 15083-2 for soft rot fungi, EN 20-2 for powder post-beetle and EN 117 and EN 118 for termites)

Organisms	Code for product	Temporary treatment of logs	Temporary treatment	Treatment of solid wood (List of standards mentioned in the tables 1 to 5 of EN 599-1) Note 1: In some conditions, ageing tests (EN 84, EN 73) or natural weathering are required (see EN 599-1) Note 2: It is highly recommended to refer to EN 599-1 to determine the tests to be done in accordance with table 1 to 5 of EN 599-1					Treatment of wood based panels[29]
				Use Class 1	Use Class 2	Use Class 3	Use Class 4	Use Class 5	
				EN 20-1 EN 20-2	EN 20-1 EN 20-2	EN 20-1 EN 20-2			
House longhorn beetle	G.31			EN 46 EN 47	EN 46 EN 47	EN 46 EN 47	EN 47	EN 47	
Common furniture beetle	G.32			EN 49-1 EN 49-2	EN 49-1 EN 49-2	EN 49-1 EN 49-2	EN 49-2	EN 49-2	
Powder post-beetle	G.33			EN 20-1 EN 20-2	EN 20-1 EN 20-2	EN 20-1 EN 20-2	EN 20-2	EN 20-2	
Fresh wood insect	G.40	No CEN standard*							
Termites	G.50			EN 118 EN 117	EN 118 EN 117	EN 118 EN 117	EN 117 EN 252	EN 117	
Marine borers	G.60							EN 275	

Blank cell: Not applicable;

* National standards available (see the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy]).

Table 29: Curative treatments: List of available standards used in wood curative treatments (based on EN 14128)

Organisms	Code for Product	Curative treatment
Brown rot fungi	G.10	
White rot fungi	G.11	
Soft rot fungi	G.12	
Sapstain fungi	G.21.1	
Blue stain fungi	G.21.2	
Mould fungi	G.22	
Wood boring beetles	G.30	
House longhorn beetle	G.31	ENV 1390
Common furniture beetle	G.32	EN 48 or EN 370
Powder post beetles	G.33	No CEN standard available
Fresh wood insect	G.40	
Termites (genus claimed)	G.50	No CEN standard available
Marine borers (genus claimed)	G.60	

*Blank cell: Not applicable

5.5.8.2.2 Preventive treatments

Most of the available data are laboratory generated and related to the organisms for which biocidal efficacy is claimed.

Field trials, although desirable in cases where the product is intended for use in the more severe service environments (e.g. in ground contact (use class 3, 4 and 5)) are considered mandatory to fulfil the minimum performance criteria, according to the tests required in the paragraphs related to the use classes. As this could lead to a significant delay before a new product could be introduced to the market, literature, monitoring or other methods provided to support the derived application rate could be accepted in case by case by the CAs (see also notes in sections 5.5.8.2.2.3 and 5.5.8.2.2.4).

The assessment of the preventive efficacy of wood preservative formulations has to be made from values derived from a relevant biological test. These values are either the actual quantitative amounts of the product established in the test as causing the appropriate level of mortality of the target organism, or they represent the threshold limits, the so-called 'toxic values'. These toxic values are two concentrations in the series used in the test, the first which just permits continued attack and the second which just prevents it.

5.5.8.2.2.1 Temporary treatments of logs (in the sawmill or in storage area)

This kind of treatment is used to prevent the degradation of logs which do not immediately have their bark removed. Indeed, some microscopic fungi (e.g. stain) infect the wood and/or some species of insects belonging to the family of *Scolytidae* and *Bostrychidae* (named "Fresh wood insect" in Table 26:) lay their eggs between the bark and the wood.

To prevent these damages, the logs may be treated with a biocidal product.

As the treatment is temporary, use class is not relevant in this case.

5.5.8.2.2.2 Temporary treatment of green timber

This kind of treatment is used for the protection of freshly felled green lumber against colonization by blue stain and other discolouring micro-organisms (often named 'sapstain' as

there are more than 200 fungi which can caused discoloration of the sapwood) and surface mould.

A technical specification (CEN/TS 15082) is available.

1 blue stain fungi and other discolouring sapwood fungi

Blue stain is caused by microscopic fungi that only infect the sapwood. They can cause blue or grey discoloration of the sapwood, but have no impact on its strength. Blue stain reduces the value of the wood.

Typical blue stain fungi are: *Ceratocystis* spp*., Ophiostoma* spp*. Aureobasidium* spp.

Typical other discolouring fungi are: *Stereum* spp*.*

In the final stage of processing in a sawmill, treatment with a biocidal product (commonly applied by dipping to prevent blue stain fungi) may be carried out.

2 moulds growing often on the wood surface

The major problems caused by moulds fungi are discoloration on surfaces, and sometimes health problems. They do not affect the strength properties of wood.

Typical mould fungal genera on wood are: *Alternaria, Aspergillus, Penicillium, Trichoderma*.

A dose rate / dipping time is part of the efficacy assessment. The label claim must mention the dose rate and the dipping time.

5.5.8.2.2.3 Treatments of solid wood (EN 599-1 Standard)

When the purpose is to protect the wood, a preventive treatment is often applied to prevent the degradation of wood by micro-organisms (for example fungi) and/or by insects (for example wood boring insects). The treatment type is related to the organisms against which the wood has to be protected and to the use class. EN 599-1 specifies what test should be done for each use class claimed.

Different target organisms may preferentially attack either softwood or hardwood. Tests must be conducted on softwood and/or on hardwood as appropriate to the target organisms and following the requirements presented in the relevant test procedures.

It must be noted that Use Class 1 requires only insecticide products and, starting from Use Class 2, products are fungicide alone or combine fungicide and insecticide activities.

It may also be noted that in some cases when a claim against only blue stain fungi is made justified exemptions are possible [30].

Use Class 1

Required data
Refer to EN 599 -1 table 1.

Data will include suitable laboratory data using treated test blocks to determine the toxic values against insects as appropriate.

Data should be presented on test blocks subjected to pre-conditioning by an evaporative ageing process (e.g. EN 73).

Test species

[30] Products which only claim protection against blue stain can be authorized for uses where exemption of the requirement for efficacy against wood destroying fungi can be justified, e.g. for wood or wood products that by their nature are not susceptible to brown rot fungi. Pure anti-blue stain products may not be used together with product against wood destroying fungi to prevent double treatment of two fungicides.

The test species used will depend upon the label claims and will include as a minimum the brown rot fungi and insects basidiomycetes and beetles spp. if appropriate (as in for Use Class 1. Use Class 1 products are only insecticides.

Products used as wood preservatives with only insecticide activity can be authorised for preventive use only in UC1. For UC2 and higher classes, efficacy against brown rot fungi basidiomycetes must be demonstrated as a minimal requirement. This clarification (of interpretation of test species) should be considered to be effective immediately (and applying to on-going/past assessments) and not subject to the standard transitional period of 2 years for new guidance."

The insect species tested will depend on whether a general or a specific efficacy claim is made. Data should demonstrate activity against one or more of the following specific insects as indicator species: *Hylotrupes bajulus, Anobium punctatum, Lyctus brunneus*, and where appropriate, termites.

Note

CAs should evaluate the available data to determine whether they are sufficient for label claims as follows:

a) for general claims against "wood boring beetles"[31]

All relevant beetle species (*Hylotrupes bajulus, Anobium punctatum* and *Lyctus brunneus*) should be tested except if data (relevant and robust literature data where the materials and methods are detailed; certification data[32] on a case by case basis) are provided which demonstrate that one of the targets is the less sensitive or that the product has an equivalent activity against all beetle species (refer to EN599-1:2014, section 5.2.3)

b) for claims against a specific beetle species

If claims against individual beetle species are detailed on a product label, then suitable efficacy data against those named target pests will be required.

c) for claims against termites

Some data on efficacy against termites will only be required when the product is to be marketed for use as a termiticidal product or where local requirements demand such activity.

For a product claiming activity against termites, suitable data demonstrating preventive efficacy against a European *Reticulitermes* species will be required.

For a product claiming efficacy against overseas tropical termites, suitable data demonstrating preventive efficacy against relevant species will be required.

Use Class 2

Required data
Refer to EN 599-1:2009 table 2.

Data will include suitable laboratory data using treated test blocks to determine the toxic values against the fungi and insects as appropriate.

Test species
The test species used will depend upon the label claims and will include as a minimum the brown rot fungi and insects if appropriate (as in Use Class 1).

Note

The CAs evaluate the available data to determine if they are sufficient for label claims as follows:

a) For claims against wood rotting fungi the following data have to be available:

Suitable laboratory data demonstrating efficacy against brown rot fungi after ageing test in accordance with EN 73.

b) For claims against wood discolouring fungi the following data have to be available:

- Suitable laboratory data on the protective efficacy of the product against blue stain in service after ageing test in accordance with EN 73 or after a natural or artificial weathering cycle as given in EN 152;
- The application process used in the tests (i.e. whether by superficial or penetrative treatment) has to be in accordance with label claims.

c) For claims against insect pests the following data have to be available:

As outlined in Use Class 1.

[31] This correction has been made for an error in drafting and should be considered to be effective immediately and not subject to the standard transitional period of 2 years for new guidance.

[32] This certification ensures that products are fit for purpose and defines a capacity in the use of products taking into account among others the durability in the function (efficiency of the treatment). The efficacy part of the certification scheme is (in France) generated according the requirement of the EN 599.

Use Class 3

Required data
Refer to EN 599-1:2009 table 3a and table 3b.

Data will include suitable laboratory data using treated test blocks to determine the toxic values against the fungi and insects as appropriate.

Test species
The test species used will depend upon the label claims and will include as a minimum the brown rot fungi and insects if appropriate (as in Use Class 1).

Note

The CAs should evaluate the available data to determine if they are sufficient for claims matrix as follows:

a) For claims against wood rotting fungi, the following data have to be available:

- Suitable laboratory tests as outlined for Use Class 2 and in addition, the efficacy will be demonstrated following preconditioning of the treated test blocks by a suitable leaching procedure according to EN 84

b) For claims against wood discolouring fungi the following data have to be available:

- Suitable laboratory data on the protective efficacy of the product against blue stain in service after a natural weathering or an artificial weathering as given in EN 152.
- The application process used in the tests (i.e. whether by superficial or penetrative treatment) should be in accordance with label claims.

c) For claims against insect pests (if relevant) the following data have to be available:

As outlined in Use Class 1, and in addition the efficacy will be demonstrated following pre-conditioning of the treated test blocks by a suitable leaching procedure according to EN 84 if technically possible (i.e. this is not the case for EN 20-1 and 20-2 due to methodological constraints).

According to EN599-1 field trial results, according to EN330 may be used by the applicant instead of certain EN 113 test results, after EN 84 leaching test to derive the brown rot fungi. They are not needed to derive the minimum retention requirements.

Moreover EN 330 may be used as an alternative to basidiomycetes laboratory tests (EN 113 + EN 84) for product under coating.

Use Class 4

Required data
Refer to EN 599-1:2009 table 4.
Data will include suitable laboratory data using treated test blocks to determine the toxic values against the fungi and insects as appropriate. In this situation available data should only include application of the preservative by penetrative treatments.

Test species
Test species used will depend upon the label claims and will likely include the following target organisms: brown and white rot fungi, soft rot micro-fungi and if relevant to label claims, blue stain fungi and insects as appropriate.

Note

The CAs should evaluate the available data to determine if they are sufficient for matrix claims as follows:

a) For claims against wood rotting fungi, the following data have to be available

- Suitable laboratory data as outlined for Use Class 3 with the following supplements:
  - all laboratory data should derive from impregnated treated test blocks (i.e. a penetrative treatment) with the test formulation to determine the toxic values against both brown and white rot fungi separately;
  - a suitable laboratory test to determine the toxic efficacy against soft rot fungi and other soil inhabiting micro-organisms is required;

b) For claims against wood discolouring fungi, the following data have to be available:

- A suitable laboratory test determining the protective efficacy of the product against blue

stain for wood in service as given in EN 152.

c) For claims against insect pests, the following data have to be available:

- As outlined for Use Class 1 and in addition, efficacy will be demonstrated following pre-conditioning of the treated test blocks by a suitable procedure according to EN 73 and to EN 84 separately).

In Use Class 4 data (e.g. EN 252, literature, monitoring or other methods) will be provided to support the derived application rate.

Use Class 5

Required data
Refer to EN 599-1 table 5.

The principal agent of decay in this situation is the marine borers. Therefore in this Use Class available data must include evidence of efficacy in a relevant marine field trial carried out for a minimum of 5 years (e.g. to EN 275 or an equivalent test).

The decay in this situation by basidiomycetes fungi does occur but marine soft rot fungi are more common causing surface softening of timber. Assessment of products against marine fungi is not normally conducted using routinely laboratory tests because of the difficulties for providing conditions which appropriately model the marine environment. There is, at present, not a recognised standard laboratory test for assessment of timber intended for use in salt water.

Test species
Test species used will depend upon the label claims. The principal agent of decay in the marine environment is the marine borers although claims against fungi can also be made.

The CAs evaluate the data to determine if they are sufficient for label claims as follows:

For claims against wood rotting fungi and marine borers, the following data have to be available:

• For fungi available data as outlined in Use Class 4 as a surrogate has to be acceptable.

• For marine borers, a relevant marine field trial data has to be carried out for a minimum of 5 years according to EN 275

5.5.8.2.2.4 Treatments of wood-based panels

The biocidal treatment of wood-based panels is achieved either during or after the manufacturing process.

During the manufacturing process, product can be included into the glue prior to application or directly by wood treatment.

The evaluation of the durability of wood-based panels against brown rot fungi and white rot fungi should be carried out according to the ENV 12038 test method.

There is no specific standardized methodology allowing the evaluation of the resistance of treated wood-based panels against soft rot or insects such as *Lyctus* spp. or termites. However, some of the existing standards usually applied to solid wood can be adapted to the evaluation of wood-based panels: CEN/TS 15083-2 (natural durability to soft rot fungi), EN 20-2 (*Lyctus* spp.), EN 117 and EN 118 (termites).

For post-manufacturing treatment, product can be applied by using a surface application process or pressure process.

In that case, the EN 599-1 is appropriate for determining the retention of post manufacture treatment.

5.5.8.2.2.5 Barrier treatment against *Serpula lacrymans*

The dry rot fungus (*Serpula lacrymans* = true dry rot fungus) occurs in buildings, causing brown rot in timber. The fungus can develop at relatively low wood moisture contents and is able to penetrate damp masonry over long distances in order to infect further timber or to develop its fruit-bodies.

In general, in case of an infestation of *Serpula lacrymans*, the infected wood is cut away. To prevent the infection of the new placed wood with fungi coming from the surrounding masonry, a curative treatment against dry rot in walls (mortar) will result in creating a 'preventive' barrier in / on walls hindering the fungus to grow through.

There is a specific Technical Specification (CEN/TS 12404) for determining the performance of a preservative applied to the upper surface of the mortar in preventing the growth of dry rot through the treated mortar when exposed to the fungus. This method is only applicable to masonry fungicides applied as a true solution of preservative. It is not applicable to rods, pastes and other similar preservative types. This method is applicable to preservatives applied to masonry by brushing, spraying and/or injection techniques or mixed into rendering and plastering mortar for masonry.

5.5.8.2.2.6 Determination of preventive product application rate with regard to service life

The evaluation of PT8 products efficacy is based on the retention of the product as determined in standard test methods, e.g. according to standards listed in EN 599-1. The values determined in this way are critical values (CV's) for a particular formulation. The application rates derived from the CV's are deemed to provide only a baseline efficacy and no conclusion on service life can be made. Indeed, neither is the term service life an absolute measure and no uniform mathematical model exists to derive such from CV's, nor is determination / claim of a distinct service life part of the BPR. Estimation of service life (ESL) is based on the assumption, that different parameters have an impact on the service life of wood. This is explained in ISO 1586-1 and ISO 15686-2.

An estimated service life of wooden products is influenced e.g. by local exposure conditions, maintenance, consumer expectation and long term experiences from field testing or industrial experiences. This can provide justification for setting higher or lower retention rates as derived from CV's only.

Because the concept of ESL is not part of the BPR and claims for a specific service life is consequently solely the applicant's responsibility, the applicant must have the right to apply for lower or higher retentions than just the CV up to the retention rate which is limited by the human health and environmental risk assessments.

In order to support his claim, for UC3 claims, the applicant should submit data from e.g. literature, EN 330. For UC4, the applicant will provide, EN 252 (applicable to UC4 claims) and/or other methods for justification.

Particular specification for use class 4:

The field trials sites (minimum two) or the data extracted from literature must be representative for climatic zones with regards to the markets targeted by the product. The selected sites must allow the evaluation of the product's efficacy on all the biological organisms covered by the label claim.

5.5.8.2.3 Curative treatment

EN 14128 is the lead standard providing detailed insight into the minimum testing requirements for wood preservatives claiming curative activity. It must be noted, that testing standards concerning PT8 products are only available for testing against wood boring insects

It is important to understand that conducting curative treatments may comprise series/combinations of different steps and application methods/techniques in order to achieve the desired result and quite often result in providing preventive and curative efficacy at the same time.

5.5.8.2.3.1 Wood boring insects

Data required to support label claims for curative efficacy may include some tests generated using existing EN standards for the relevant beetle species or other alternative supporting data.

A number of EN standard tests exist for curative treatments for insecticides against *Hylotrupes bajulus* (ENV 1390) and *Anobium punctatum* (EN 48).The curative activity against *Lyctus* is not tested separately but is derived from results from testing against *Anobium punctatum* and *Hylotrupes bajulus.*

5.5.8.2.3.2 Termites

The control of termites enters into the scope of the PT8 and the PT18 depending on the use of the product. The definition of the product type is related to the use/mode of application of the product.

The reader is also invited to refer to the PT18 efficacy (section 5.6.4).

The curative treatments against termites are designed most of the time to kill the termite colony and prevent degradation of wood.

We can distinguish treatment applied to wood, for example treatment of art furniture, wood rubble from treatment applied to other support than wood for example soil or masonry.

If the product is applied on wood, then this product is covered by the requirement of the PT8. If the product is applied on another support than wood then it is covered by PT18.

We can distinguish three groups of termites:

- **Drywood termites (Cryptotermes, Kalotermes):** Drywood termites live inside of the wood which is attacked. The curative treatments applied to the wood consequently destroy the entire colony.
- **Subterranean termites (Reticulitermes, Coptotermes, Heterotermes):** The core of the subterranean termite colony is located in the soil. Termite workers built tunnels to reach wood and destroy it. The treatment applied on infested wood kills the termites present inside of the wood but not the other members of the colony.
- **Tree termites (*Nasutitermes*):** Tree termites built epigeous (above-ground) nests, frequently on living trees. As a part of the colony has a subterranean location, termites infestations of wood in building may originate either from the nestmates located in the ground or in the epigeous nests. The treatment applied on infested wood kills the termites presents inside the wood but not the other members of the colony.

5.5.8.2.3.3 Fungi

Any claims for curative activity against wood rotting fungi will be supported by suitable efficacy data. No EN standard test protocols presently exist for curative treatments applied to wood. In general, as curative treatment, the infected wood is cut away.

In all cases CAs evaluate the data available to determine if they are sufficient for supporting the label claims.

5.5.8.2.4 Resistance

Information on resistance and the likelihood of its development is required for BPR Annex I inclusion and is also demanded for product authorisation.

At this point, no target organism resistance in field of chemical wood preservatives is known.

More information on resistance can be found in Chapter 6.2 of this TNsG on Product Evaluation, in the Chapter 10 on the TNsG on the BPR Annex I inclusion and on the website of the Insecticide Resistance Action Committee and the Fungicide Resistance Action Committee (FRAC: http://www.frac.info).

5.5.8.3 Biological re-testing after changing the product formulation

While EN599-1 and EN 14128 provide the baseline for the testing requirements of new products, the corresponding annexes to both standards provide guidance on testing requirements when a formulation variation is caused by the addition, the substitution or removal of an active substance. Not all changes are subjected to re-testing and the informative sections of the standards do allow the consideration and taking into account of other data on a case by case expert judgment basis without additional testing. These data sources are not defined in detail but could include:

- Literature data;
- Certification of the product by recognised national quality scheme systems e.g. CTBP+RAL;
- National registrations;
- Others.

For any other changes in the formulation, refer to the informative annex A of EN599-1 and EN 14128. An explanation of Annex A of EN599-1 can be found in Appendix 12**.**

5.5.9 PT9 Fibre, rubber and polymerised materials preservatives

The text for this section is under section 5.5.7 with PT7.

5.5.10 PT10 Construction material preservatives

Please refer to the General sections 1-3 and the Preservatives general sections (i.e. 5.5.1-5.5.3) of this guidance.

5.5.11 PT11 Preservatives for liquid-cooling and processing systems

5.5.11.1 Introduction

This guidance describes the nature and extent of data which should be available to support the label claims for biocidal products within the main group 2 preservatives, product type 11, as described in Annex V of the BPR for:

MAIN GROUP 2: PRESERVATIVES

Product-type 11 (preservative for liquid-cooling and processing systems)

Products used for the preservation of water or other liquids used in cooling and processing systems by the control of harmful organisms such as microbes, algae, and mussels.

Examples: usages area may include but not limited to, once-through, open and closed cooling systems, non-food pasteurisers/sterilisers/can warmers, industrial process water (including marine water), humidifiers, fountain solution, and rinse bath.

Some uses are borderline between product types 11 and 12 and also with other product types. For uses for which PT allocation is not clear the relevant CA should be consulted.

For products with uses that do not fit in one of the described uses, it is up to the applicant to demonstrate efficacy in an appropriate way. In that case, a justification for the relevance of the tests used should be provided. The general guidance principles laid out in sections 5.5.1-5.5.5 of this guidance need to be respected. If needed, the test design should be discussed with and agreed upon by the CA before testing takes place. The evaluation will be done on a case-by-case basis by the CAs.

5.5.11.2 Preservation of cooling systems

5.5.11.2.1 Introduction

Cooling systems are based on thermodynamic principles and are designed to promote the efficient transfer of heat between process and coolant. The exchange of heat between the process medium and coolant is enhanced by the heat exchangers. From the heat exchangers, the coolant transports the heat into the environment. Several kinds of cooling systems are described below:

- In **once-through cooling systems**, commonly applied to large capacity installations, water is pumped from a source (e.g. river, lake, sea, or estuary). After passing heat exchangers or condensers, the heated water is discharged directly back into the surface water. In some cases, a once-through cooling system can be combined with a cooling tower to cool the water before it is discharged back to the surface water. In this kind of tower, no recirculation of the water is performed.
- In **open recirculating cooling systems** cooling water is cooled down by contact with an airstream in a cooling tower. They are equipped with devices (tower fill) to enhance the air/water contact. The airflow can be created by mechanical draught using fans or by natural draught. The mechanical draught towers are used widely for small and large capacities. Natural draught towers mostly are applied for large capacities (e.g. power industry). Cooling towers (wet cooling towers) operate on the principle of evaporative cooling. The efficiency of the process is correlated to the dryness of the air. The warm water is cooled to a lower temperature by spraying it through a flow of air. A small portion of the water is evaporated inducing a decrease of the temperature of the

remaining water. In this kind of system, recirculation of the water is performed. Due to evaporation, the concentration of salts increases and therefore circulating cooling systems need to discharge water continuously (blowdown). Water losses due to evaporation and blowdown are compensated by the inlet of fresh water (make-up water). Consequently, biocides need to be dosed at regular intervals or continuously. The system sizes can vary vastly from large systems in industrial applications to small systems located on roofs to cool buildings.

- **Closed systems** are widely used in industry from smaller capacities. In closed systems (also named closed-loop cooling systems), the coolant fluid which may be water or process medium circulates inside tubes or coils and is not in open contact with the atmospheric air: The tubes or coils are cooled, in turn cooling the substance they contain. In a dry closed-loop, the tubes or coils are cooled with air. Coils can be equipped with fins that enlarge the cooling surface and consequently increase the cooling effect. In a closed-loop evaporative system, the closed-loop heat exchanger is placed into a cooling tower, the tubes or coils are sprayed with water and cooled then by evaporation.

In addition for recirculating open and closed cooling systems treatment occurs also for the make-up water: The raw water used for cooling systems may have different origins. City water is often used in tertiary, agri-food, and pharmaceutical activities. Heavy industries may also use well or surface waters. Sea water can also be used; macro-fouling issues are common in this case. It is also possible to use recycled process waters, which may need a specific treatment. Therefore, raw water may need treatment before entering the cooling system as make-up water.

5.5.11.2.2 Purpose of the biocidal treatment

The purpose of the biocidal treatment is to maintain the function of process water, the efficiency of heat exchange and the integrity of assets (e.g. protect the cooling system from adverse effects, e.g. protection of corrosion of pipes, valves and filter blocking) and to keep the risks posed by harmful organisms under control. It can be achieved either by maintaining or reducing the population of micro-organisms below a defined limit by preventive or curative use of biocides, respectively.

The microbial issues in cooling systems are related to the occurrence of different species of harmful organisms in the cooling water (planktonic) or on the parts of the system that are in contact with the cooling water (biofilm). These issues are often interconnected. Any solid deposit, whether it is corrosion debris, mineral scale, FeS or biomass, can be a niche for micro-organisms, promote microbial activity and lead to the formation of galvanic cells and localised corrosion.

Microbial contamination (e.g. biofilm) can occur on any wet surfaces within the cooling system and biological fouling reduces the efficiency of the heat exchange. Fouled systems may also be a human health concern due to the presence of e.g. *Legionella pneumophila*. Other pathogens like *Naegleria fowleri* or mycobacteria may also grow in fouled systems. Growth of *Legionella* is typically enhanced by conditions present in cooling towers such as a temperature range from 20 to 45°C (Tison *et al.* 1981, Fliermans *et al.* 1981, Habich and Müller, 1988), a pH range from 6 to 8, presence of fouling (Fields, 2002, Tison *et al.* 1981) and of amoebae and algae.

Note that national legal requirements for *Legionella* and amoebae control may exist in member states. These requirements do not fall under the biocides legislation and are not considered in this guidance. An overview of the legal requirements in different MS can be found in a report of the European Agency for Safety and Health at Work (OSHA)[33]. For amoeba, limits are depending on the type of industry.

5.5.11.2.3 Mode of application

Different types of applications of biocidal products can be used: e.g. manual dosing, dosing with a pump, special dissolving/dosing device for solid biocides, or a generator for *in situ* generation of a biocidal active substance. Furthermore, several dosing regimens can be applied.

[33] https://osha.europa.eu/en/publications/legionella-and-legionnaires-disease-policy-overview
https://osha.europa.eu/en/publications/factsheet-100-legionella-and-legionnaires-disease-european-policies-and-good-practices/view

Preventive treatment

Continuous application: biocide is injected continuously, or at a frequency that enables the maintenance of the concentration of biocide in a defined range.

Batch application: biocide is injected regularly, or at a frequency that does not ensure the maintenance of a constant efficacious concentration in the system. The concentration of the biocide is maximal after the injection and decreases to a minimum concentration with time.

Curative treatment

Biocide is injected as a shock dosage at a concentration, which is usually higher than preventive treatment, in response to unexpected and/or heavy microbiological growth.

Preventive and curative treatment take place most of the time on-line. However, in some cases, curative treatment occurs off-line (e.g. in case of a strong *Legionella* contamination. See the definition for off-line system in Appendix 26). It should be noted that the application of a biocide during an off-line treatment, means that the biocide is still distributed in the system by water re-circulation.

Due to the specificity of each system (in terms of volume, capacity, rate of evaporation, blowdown, etc.) and the possible degradation/accumulation of biocides linked to the holding time index, the exact effective dose within the authorised dose range and the frequency of dosing cannot be validated beforehand. The following instruction for use should be added to the SPC:

"*It is the responsibility of the end-user to determine the effective dose on-site (e.g. by chemical or microbiological tests) for the specific location/system to ensure that the system is efficacious under the use conditions. If needed, the manufacturer of the preservative product can be consulted.*"

5.5.11.2.4 Data requirements

The efficacy data presented in the dossier should be representative for the use claimed, i.e. consistent with the microbial contamination related to the type of cooling system and with the mode of application/regimes.

Preventive action

For preventive action, the biocidal treatment is applied to the matrix and then the challenge consortium is added. A mixed consortium of a relatively small size is employed to both model a clean system becoming 'inoculated' through environmental contamination and to ensure that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved in the untreated specimens. The samples are incubated for a contact time appropriate for the biocide being tested and then analysed. The treatment is considered effective when growth in the matrix and the formation of biofilm on the coupons immersed in it is prevented in the treated specimens.

Curative action

For curative action, multiple samples of a matrix are inoculated with a mixed consortium of relatively small size so that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved. Once growth has been achieved samples selected at random are either treated with the biocide or left untreated (or treated with water/the solvent used for the biocide treatment). The samples are incubated for a contact time appropriate for the biocide being tested and then analysed. The objective is that no decrease in the size of the populations should be observed in the untreated samples during this contact time, but a decrease (curative effect) should be observed in the treated samples.

Test methods

Preventive treatment

For bacteria (including *L. pneumophila*), algae, yeasts, or fungi: laboratory tests should be performed according to methods such as:

- IBRG FFG 19-006.2; or
- modified ASTM E645 where the level of inoculum is lowered, and the concentration of nutrients (yeast extract) is modified in order to show growth in the controls.

These tests should be performed in cooling water extracted from the field or in synthetic model water[34] with relevant reference strains of the target organisms.

For mycobacteria: a similar approach than the one presented for bacteria can be carried out.

For biofilm: laboratory tests should be performed according to IBRG FFG 19-007.2.

For a specific claim against amoeba: currently no standardised method for preventive efficacy against amoebae exists. The applicant should suggest an appropriate test strategy and discuss it with the CA.

For a specific claim against Mollusca: The efficacy demonstration should be based on:

- a laboratory test with *Mytilus sp*. to determine the impact of the treatment (e.g. mussel settlement, growth rate, filtration rate, oxygen consumption, foot activity, byssus thread production); and
- field monitoring data demonstrating the efficacy of the product (e.g. prevent the mussel larvae settlement and/or prevention of the growth) in comparison to an untreated system or in comparison the part of the system before the biocide dosing point to the parts after the dosing point or with the same system with and without treatment over the monitoring period (at least during one swarming season).

Curative treatment

Nb: In case an off-line disinfection is required, e.g. due to Legionella pneumophila contamination: the use refers to a PT2 application. (See PT2 section)

For bacteria, algae, mycobacteria, yeasts or fungi: For liquid-cooling systems, an appropriate laboratory test, such as based on ASTM E645 or IBRG FFG21-008 (using aerobic organisms), is required with cooling water extracted from the field, tested with relevant reference strains of the target organisms or with a synthetic model water[34] with reference strains.

For biofilm (on-line): For sessile bacteria, laboratory simulated-use tests should be conducted to demonstrate the ability of the product to control the biofilm under static conditions or under flow conditions depending on the claim. This test should be performed according to an adapted method such as IBRG FFG21-008 (for aerobic use conditions) or IBRG FFG21-011 (for anaerobic use conditions) in two steps:

1. the specimens (coupons of representative material) are immersed in the model matrix inoculated with a consortium of bacteria and cultured under representative conditions.
2. after the biofilm is established on the coupons, the biocidal product is added at the application rate to the matrix. Efficacy is assessed after the contact time, according to criteria set in the section Acceptance criteria. The level of the contamination should remain stable or increase in the untreated controls during the contact time. Both untreated control and treated samples should be performed at least in triplicates.

For a specific claim against amoebae: For the demonstration of the efficacy against amoebae, the efficacy should be based on a laboratory test and monitoring data; or based on a simulated-use test. The laboratory test should be performed with *Acanthamoeba* species to determine the microbial inactivation, loss of cultivability as a function of time Ct (concentration × exposure time ($Ct_{99.9\%,}$ e.g. lg reduction of 3)) during the preservative exposure (mg·min/l) (Chick 1908)[35].

The simulated-use test is designed to mimic a worst-case scenario and practical use situations. The test should be relevant to the use and label claim. A control treatment without biocide has to be included. At least three replications of the treatment experiments should be provided.

If properly justified, deviations to the protocol presented below can be accepted.

The simulated-use test should be performed in a pilot-scale functioning cooling tower system (Liu *et al.* 2011)[35]. The system should be constructed using relevant materials found in

[34]The reconstituted water should represent the characteristics of the application (pH, organic matter content, hardness, etc.). However, this is not always possible due to the high variability in the quality of the different water sources and the mode of operation. In these cases, the following water quality can be used as a testing matrix: pH 8 (+/-0.2), Ca > 300 mg/L, total alkalinity > 400 mg/L $CaCO_3$), DOC (dissolved organic compound) at a maximum level of 100 mg/L. Other alternative synthetic water such as described in the IBRG method can be used if appropriate.

[35] References in Appendix 25

conventional full-size cooling tower systems. A justification should be provided clarifying the rationale for the chosen materials. The following parameters should be assessed, reported, and chosen according to the claim:

- recirculated water should be kept at an average temperature range from 25 to 30 °C. If other temperature ranges are necessary due to the specific nature of the respective claim, a scientifically sound justification must be provided by the applicant;
- flow rate;
- air intake rate;
- the source of the used make-up water source, and relevant treatment of the make-up water should be described, interference with the tested biocidal treatment in the cooling system has to be avoided;
- temperature in the cool-water basin.

Free-living amoeba has been described to act as a vector for pathogenic microorganisms and to act as pathogens as well (Canals *et al*. 2015, Scheikl *et al.* 2016, Greub and Raoult 2004)[35]. Amoebae may be present as trophozoites or as cysts which are more resistant to biocidal treatment than the free moving trophozoites. Furthermore, trophozoites or cysts may be present in bacterial biofilms in the cooling system. As test organism trophozoites of *Acanthamoeba* spp. should be used for a general claim against amoebae. If the cyst form is claimed, it should also be tested.

To assess the efficacy of biocidal treatment cysts at a concentration of 10^6/l should be used. At least three samples of 1 litre should be taken to assess the remaining inoculum in the treated systems. After the claimed treatment at least a lg reduction of 3 should be achieved. The method used to assess the number of viable amoebae should be justified and described in detail (e.g. filtration parameters). Two different assessment methods should be used obligatory to allow a semi-quantitative assessment (e.g. MPN, qPRC live dead, cell sorting). In the untreated control set-up, the number of viable amoebae should not decrease below 10^4 of the initial inoculum after the claimed test time.

Field monitoring data from a system, where the use of the product is consistent with the label claim, can be submitted. The efficacy can be demonstrated in the treated system in comparison to an untreated system or in comparison between a part of the system prior to the biocide dosing to a part after the dosing or between the same system with and without treatment. If no guideline is available for a specific use of a product, or if guidelines are not suitable, the applicant may use other methods (such as intra-company Standard Operating Procedures), where the studies are scientifically robust, well-reported and provide a clear answer to the question. The general requirements for preservative testing laid out in sections 5.5.1-5.5.5 of this guidance should be respected. In addition, the test methods used, together with the test conditions, should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with modifications to make the guideline more suitable for the specific product or use conditions, is also possible.

When standards are modified or non-standard methods are used, a scientific justification has to be presented. Furthermore, such deviations should be agreed upon with the CA before conducting the tests. In all cases, a scientifically robust test protocol should be agreed on with the evaluating CA before testing.

In a specific case of highly reactive active substance or with no long-lasting effect (e.g. due to removal by quick reaction with organic matter or self-degradation, justified by scientific evidence), applied only by continuous dosing, continuous active substance dosing can be acceptable in the tests.

Test organisms

The minimum mandatory and optional test organisms for the different target sites and in accordance with the respective claims are presented in Table 30 below. Every additional target organism claimed (not presented in this table) should be justified and supported by suitable efficacy data. For the demonstration of the efficacy against bacteria, yeasts, fungi and mycobacteria, tests performed with consortia of strains presented in Table 30 are required. The growth of each target organism group should be assessed separately. For a general claim against algae, both cyanobacteria and (green) algae should be tested in the consortium. Both can also be claimed separately. Regarding the demonstration of the efficacy against *Mollusca*,

test(s) against mussels should be performed. For a general claim against amoeba for the demonstration of efficacy, test(s) should be performed with trophozoites of *Acanthamoeba spp*. If efficacy is claimed against the cyst form, cysts should be tested.

Test conditions

Conditions should be representative for the uses claimed (oxic/anoxic). Note that for *Desulfovibrio sp.* anoxic condition in the test is required.

Application rate

The application rates of the products can be adapted in connection to the chemistry of the water (fresh water/salt water), the hardness of the water, the organic matter content, the use of scaling and corrosion inhibitors, the hydraulic cycle time (when relevant) and the holding time index (evaporation and blowdown; see definitions in Appendix 26). Such parameters should be taken into account when generating the efficacy data in connection to the uses claimed. Efficacy testing should focus on using critical parameters representative of the field of use, so that an indication of efficacy in variable installations is provided. Every installation to be treated has its own characteristics. The end-user should ensure the efficacy of the biocidal treatment by conducting microbial analysis.

In case of dose ranges, efficacy should be demonstrated at the minimum intended dose. Nevertheless, the following instruction for use should be added to the SPC:

"*It is the responsibility of the end-user to determine the effective dose on-site (e.g. by chemical or microbiological tests) for the specific location/system to ensure that the system is efficacious under the use conditions. If needed, the manufacturer of the preservative product can be consulted.*"

Contact time

For preventive treatment, the contact time is not relevant and will not be declared in the SPC of the product. Test durations for preventive treatment should be chosen in accordance with the respective claims and with validity requirements. For the continuous or frequent batch application of the biocidal product, the test duration should provide sufficient time for the untreated control to grow. Multiple dosing or continuous dosing of the biocidal product is acceptable, if necessary, in order to reflect the claimed conditions of use; in such cases, water/solvent should be dosed in the controls accordingly. In case of a low-frequency batch application, i.e. when the application interval of the biocidal product is longer than the time required to achieve growth in controls, the test duration should be as long as the claimed application interval.
For curative treatment, information on contact time should be included in the SPC. The duration of curative efficacy testing should reflect the label claims. In laboratory tests for batch applications, normally these contact times should be applied:

- for a fast-acting product an exposure time ranged from 1 to 3 hours;
- for a slow-acting product an exposure time ranged from 6 to 24 hours.

For specific cases, the contact time should start from the time when the concentration has reached a plateau level in the system (e.g. in a recirculating cooling tower, when a curative treatment is applied, the efficient curative concentration is not immediately reached in all parts of the system. Another example is active substances such as ozone, which requires a period before its concentration reaches an equilibrium in the system. The contact time should start from that moment).

In the SPC for both preventive and curative treatments, the frequency of applications (per day or per week) should be mentioned.

Temperature

Regarding the temperature, an average temperature ranging from 20 to 30°C should be used. This temperature range is representative of the temperatures met in cooling systems where problems can occur. This range also allows the growth of microorganisms in the laboratory. If other temperature ranges are necessary due to the specific nature of the respective claim, a scientifically sound justification must be provided by the applicant.

5.5.11.2.5 Acceptance criteria

The required tests should be performed (using the required test organisms and test conditions), in accordance with the general test guidelines laid out in sections 5.5.1-5.5.5 of this guidance. Efficacy criteria should be achieved for the claimed contact time. The following criteria are usually the minimum requirements that need to be demonstrated.

Preventive treatment

See section 5.5.11.2.4 for a description of the preventive action of a biocide.

Validity of the studies: regardless of the method used, growth of the test organisms must be demonstrated in the untreated inoculated controls. Growth means an increase over the recovery directly after inoculation which is statistically significant and greater than 0.5 lg[36].

In justified cases, it can also be acceptable to show the degradation of the matrix instead. In that case, cooling fluid from the field should be used instead of synthetic cooling water.

Efficacy criteria for all the target organisms: compared to the recovery directly after inoculation of the biocide-treated inoculated samples, no growth of the target organisms should occur.

Curative treatment

See section 5.5.11.2.4 for a description of the curative action of a biocide.

Validity of the study: In the inoculated vessels, growth does not need to be shown in the untreated control, nevertheless the initial size of the inoculum should be sufficiently high to show a reduction. Over the course of treatment, the number of target organisms in the untreated inoculated controls must remain stable or increase compared to the number directly before treatment. A decrease of the level of inoculum in the control in this period is acceptable only if it is not statistically significant and not greater than 50%.

Efficacy criteria: The reduction is defined by the comparison of the level of inoculum in the treated sample at t=0 to the level of inoculum in the treated sample at the end of the contact time:

- for bacteria (*including Legionella*): 3 lg reduction is the minimum level of performance;
- for yeasts and fungi: 2 lg reduction is the minimum level of performance;
- for biofilm: 2 lg reduction is the minimum level of performance;
- for amoebae: the application rate claimed, and the contact time should be consistent with the $Ct_{99.9}$ value derived from the laboratory test. If another method is used, 3 lg reduction should be shown;
- for mycobacteria and algae, a reduction of 95% is the minimum level of performance. For algae, the reduction can be measured in viable cells or in the concentration of chlorophylla.

[36] Student test with a p-value <0.05 (95 % confidence level) is highly recommended, p <0.1 (90% confidence level), if justified (e.g. identifying outliers, or lowest concentration with a biocide showing growth). For filamentous fungi, different rules apply for quantification of growth. Please refer to section 5.5.4.2 of this guidance and to TAB #16 version 2.1 (WGIV2019) "Growth quantification or determination of filamentous fungi".

Table 30: Preservation of water in cooling systems: test organisms according to the claims

Uses: Preservation of cooling systems		Bacteria				Fungi		Algae		Amoeba	Mollusca	Mycobacteria
						Yeasts	Moulds					
Target site	**Purpose**	*Pseudomonas aeruginosa* + *Enterobacter cloacae* + *Aeromonas hydrophila* + *Micrococus luteus*	*Desulfovibrio* sp.	*Gallionella*	*Legionella pneumophila*	*Candida albicans* + *Rhodotorula mucilaginosa*	*Cladosporium cladosporioides* + *Penicillium purpurogenum*	*Chlorella vulgaris*	*Anabaena* spp.	*Acanthamoeba spp.*	*Mytilus* sp.	*Mycobacterium chelonae* *Mycobacterium abssessus*
Once-through cooling system without cooling tower	On-line preservation of water to maintain or reduce the population of bacteria and if appropriate other micro-organisms.	X	O	O	O	O	O	O	O	O	O	O
Once-through cooling system with cooling tower		X	O	O	O	O	O	O	O	O	O	O
Open recirculating cooling system with cooling tower		X	O	O	O	O	O	O	O	O	O	O
Closed-loop cooling systems		X	O	O	O	O	O	O	O	O	O	O
Make-up water for cooling systems		X	O	O	O	O	O	O	O	O	O	O

- X: mandatory; O: optional

5.5.11.3 Preservation of processing liquids

5.5.11.3.1 Introduction

Air conditioning and air washers with drift eliminators/scrubbers/humidifier systems:

Several air treatment systems exist, for example:

Air conditioning and air humidification through spraying water on a media (some systems use vapor for humidification). It has to be noted that some small systems with sporadic use, may not require biocidal treatment during normal operation.

Adiabatic cooling enables the cooling of air under adiabatic conditions: heat energy is neither added nor removed. Water is vaporized into the air, the heat required for evaporation is taken from the air, consequently air temperature decreases, and moisture content increases.

Water scrubbers are air pollution control devices used to remove particulate matter or gases from industrial exhaust or flue gas streams. Water streams entrain particles and pollutant gases in order to wash them out of the gas flow. *Legionella* may grow as well as other microbes of health concern and this needs to be prevented. Scrubbers have been involved in legionellosis outbreaks.

Pasteurisers, sterilisers, wash waters and conveyor lubricants

Pasteurisers: Pasteurisation is the last process applied in the production of liquid products to ensure adequate product quality and shelf life. In principle it is very simple, consisting usually of a steel tunnel with a track conveyer passing through to carry the product. The bottom of the tunnel consists of a series of tanks feeding overhead spray nozzles that are directed onto the product as it passes through. There is a gradient of temperatures along these tanks that gradually heat, hold at pasteurizing temperature, then cool the product. The gradient is designed to produce minimum thermal stress on the product and containers. In most pasteurisers, the temperatures of the zones are controlled by the addition of steam or of processing water. Biocides are needed to prevent microbial growth in the treatment systems for pasteuriser water.

Wash water in the rinse area of the tunnel washer: In tunnel washers, water from the rinse zone is reused in the wash, especially for the preservation of water in times during which the laundry process is stopped (e. g. overnight/weekends). Biocides are needed when the laundry process is stopped (e. g overnight/weekends) to preserve the rinse water and to prevent smell development in the rinse area and the effluent pit.

Conveyor lubricants: These lubricants are used to reduce the friction between the packaging materials e.g. bottles (or other materials to be transported) and the belt and to inhibit rust and corrosion, to carry away heat and to cushion impact forces on chain conveyors. The lubricants thus slow down the wear of the single parts in the chain extending its work life. Furthermore, they flush out wear debris and foreign materials. In order to keep the lubricants free from microbial contaminants that would deteriorate them leading to a failure of the chain conveyor, biocides are added.

Preservation of liquids used in closed/opened recirculating heating and associated pipework

From an efficacy point of view, closed recirculating heating systems should be considered as closed cooling systems as the conditions are similar. Closed systems are less susceptible to corrosion, scaling and biofouling than open systems. This description is also applicable to compressor cooling, air conditioning chilled water, boilers, engine jacket cooling and power supply.

5.5.11.3.2 Purpose of the biocidal treatment

Air conditioning and air washers with drift eliminators/scrubbers/humidifier systems

The purpose of the biocidal treatment is mainly to maintain (preventive action) or reduce (curative action) the population of micro-organisms (bacteria, and if appropriate yeasts or fungi) in the water used in air conditioning/air washer/humidifier, to an acceptable level in order to maintain the system clean (media, basin and nozzles) and also, depending on the case, in order to keep the risk of harmful organisms under control (e.g. *Legionella*). Every installation to be treated has its own specificities and it is up to the end user to ensure, e.g. through biological tests, the efficiency of the system.

Pasteurizers, sterilisers, wash waters and conveyor lubricants

The purpose of the biocidal treatment is to control micro-organisms by maintaining (preventive action) or reducing (curative action) the population of micro-organisms (bacteria, yeasts and fungi) in the water used in pasteurizers, tunnels washers or conveyor lubricants, to an acceptable level to keep the system clean (e.g. to prevent the clogging of the pipes).

Preservation of liquids used in closed/opened recirculating heating and associated pipework

The biocidal product can be applied in used or new structural pipes built on industrial building projects to control the growth of microorganisms in the circulating water in order to protect the installation. To prevent the degradation of installations by frost, anti-frost chemicals (e.g. glycol based) can be added to the heating fluid. Subsequently, biocidal products can be added to the system as well to prevent microbiological degradation of the anti-frost agents or other functional additives used as nutrients by the microorganisms. In that case, no curative application is expected.

5.5.11.3.3 Mode of application

Different types of applications can be used: e.g. manual dosing, addition with a dosing pump, special dissolving/dosing device for solid biocides, or a generator for *in situ* production of biocide substance.

Furthermore, several dosage regimes can be applied:

Preventive treatment

- Continuous application: biocide is injected continuously or at a frequency that enables the maintenance of the concentration of biocide in a defined range.
- Batch application: biocide(-s) is/are injected regularly or at a frequency that does not ensure the maintenance of a constant efficacious concentration in the system. The concentration of the biocide is maximal after the injection and decreases to a minimum concentration as indicated with time.

Curative treatment

- Biocide is injected as a shock dosage at a concentration, which is usually higher than preventive treatment, in response to unexpected and/or heavy microbiological growth.

Preventive and curative treatments take place most of the time on-line. In some cases, curative treatments occur off-line (e.g. in case of strong contamination). In this context, off-line is when the process cooled/heated by the processing system is not operated because of the implementation of a cleaning and/or disinfection event. As an example, stopping of the heat source or cooling fans in processing systems in order to clean and/or disinfect the system would be considered as an off-line operation. However, it should be noted that the application of a biocide during an off-line treatment, means that the biocide is still distributed in the system by water re-circulation.

Treatments are either preventive or curative, depending on the target organisms and the extent to which biofouling has already progressed.

Due to the specificity of each system (e.g. volume, capacity, rate of evaporation, blowdown) or due to the possible degradation/accumulation of biocides linked to the holding time index, the frequency of dosing cannot be validated beforehand. The following instruction for use should be added to the SPC:

"*It is the responsibility of the end-user to determine the effective dose on-site (e.g. by chemical or microbiological tests) for the specific location/system to ensure that the system is efficacious under the use conditions. If needed, the manufacturer of the preservative product can be consulted.*"

5.5.11.3.4 Data requirements

The efficacy data presented in the dossier should be representative of the uses claimed, i.e. consistent with the microbial contamination related to the use and with the mode of application/regimes.

Preventive action

For preventive action, the biocidal treatment is applied to the matrix and then the challenge consortium is added. A mixed consortium of a relatively small size is employed to both model a clean system becoming 'inoculated' through environmental contamination and to ensure that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved in the untreated specimens. The samples are incubated for a contact time appropriate for the biocide being tested and then analysed. The treatment is considered effective when growth in the matrix and the formation of biofilm on the coupons immersed in it is prevented in the treated specimens.

Curative action

For curative action, multiple samples of a matrix are inoculated with a mixed consortium of relatively small size so that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved. Once growth has been achieved samples selected at random are either treated with the biocide or left untreated (or treated with water/the solvent used for the biocide treatment). The samples are incubated for a contact time appropriate for the biocide being tested and then analysed. The objective is that no decrease in the size of the populations should be observed in the untreated samples during this contact time, but a decrease (curative effect) should be observed in the treated samples.

Test methods

Preventive treatment

For bacteria, yeasts and fungi:

A laboratory test should be performed according to methods such as:

- IBRG FFG 19-006.2 or;
- modified ASTM E645, where the level of inoculum is lowered and the concentration of nutrients (yeast extract) is modified in order to show growth in the controls.

The tests should be performed with a representative sample of water extracted from the field, or with synthetic model water[34] tested with relevant reference strains of the target organisms.

Preventive treatment for biofilm:

- laboratory tests should be performed according to IBRG FFG 19-007.2

Curative treatment

Nb: In case an off-line disinfection is required, e.g. due to Legionella pneumophila contamination: the use refers to a PT2 application (see PT2 section).

For bacteria, yeasts and fungi:

For processing systems, an appropriate laboratory test, such as based on ASTM E645 or IBRG FFG21-008 (using aerobic organisms), is required with a representative sample of water extracted from the field, tested with relevant reference strains of the target organisms, or with synthetic model water[34] with reference strains.

For biofilm: For sessile bacteria, laboratory simulated-use tests should be conducted to demonstrate the ability of the product to control the biofilm under static conditions or under flow conditions depending on the claim. This test should be performed according to an adapted method such as IBRG FFG21-008 (for aerobic use conditions) or IBRG FFG21-011 (for anaerobic use conditions) in two steps:

1. the specimens (coupons of representative material) are immersed in the model matrix inoculated with a consortium of bacteria and cultured under representative conditions.
2. after the biofilm is established on the coupons, the biocidal product is added at the application rate into the matrix. Efficacy is assessed after the contact time, according to criteria set in the section Acceptance criteria. The level of the contamination should remain stable or increase in the untreated controls during the contact time. Both untreated control and treated samples should be performed at least in triplicates.

If no guideline is available for a specific use of a product, or if guidelines are not suitable, the applicant may use other methods (such as intra-company Standard Operating Procedures), where the studies are scientifically robust, well-reported and provide a clear answer to the question. The general requirements for preservative testing laid out in sections 5.5.1-5.5.5

should be respected. In addition, the test methods used, together with the test conditions, should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with modifications to make the guideline more suitable for the specific product or use conditions, is also possible.

When standards are modified or non-standard methods are used, a scientific justification has to be presented. Furthermore, such deviations should be agreed on with the CA before performing the tests. In all cases, a scientifically robust test protocol should be agreed on with the evaluating CA before testing.

In the specific case of highly reactive active substance or with no long-lasting effect (e.g. due to removal by quick reaction with organics or self-degradation), applied only by continuous dosing, continuous active substance dosing can be acceptable in the tests.

Test organisms

The minimum mandatory and optional test organisms for the different target sites and accordance with the respective claims are presented in Table 31 below. Every additional target organism (not presented in this table) should be justified and supported by suitable efficacy data. For the demonstration of the efficacy against bacteria, yeasts and fungi, tests performed with consortia of the strains presented in Table 31 are required. The growth of each target organism group should be assessed separately.

Test conditions

Application rate:

The application rates of the products can be adapted in connection to the chemistry of the water (hardness, organic matter content, use of scaling and corrosion inhibitors), the hydraulic cycle time (when relevant) and the holding time index (evaporation and blowdown). These parameters should be taken into account when generating the efficacy data in connection to the uses claimed. Efficacy testing should focus on using critical parameters representative of the field of use, so that an indication of efficacy in variable installations is provided. Every installation to be treated has its own characteristics. The end-user should ensure the efficacy of the biocidal treatment by conducting microbial analysis.

In case of dose ranges, efficacy should be demonstrated at the minimum intended dose. Nevertheless, the following instruction of use should be added to the SPC:

"*It is the responsibility of the end-user to determine the effective dose on-site (e.g. by chemical or microbiological tests) for the specific location/system to ensure that the system is efficacious under the use conditions. If needed, the manufacturer of the preservative product can be consulted.*"

Contact time:

For preventive treatment, the contact time is not relevant and will not be declared in the SPC of the product. Test durations for preventive treatment should be chosen in accordance with the respective claims and with validity requirements.

For the continuous or frequent batch application of the biocidal product, the test duration should provide sufficient time for the untreated control to grow. Multiple dosing or continuous dosing of the biocidal product is acceptable, if necessary, in order to reflect the claimed conditions of use; in such cases, water/solvent should be dosed in the controls accordingly.

In case of a low-frequency batch application, i.e. when the application interval of the biocidal product is longer than the time required to achieve growth in controls, the test duration should be as long as the claimed application interval.

For curative treatment, information on contact times should be included in the SPC. The duration of curative efficacy testing should reflect the label claims.

In laboratory tests, for batch application, normally these contact times should be applied:

- for a fast-acting product an exposure time ranged from 1 to 3 hours;
- for a slow-acting product an exposure time ranged from 6 to 24 hours.

For specific cases, the contact time should start from the time when the concentration has reached a plateau level in the system (e.g. in a recirculating process system when a curative treatment is applied, the efficient curative concentration is not immediately reached in all parts

of the system. Another example is for active substances such as ozone, which requires a period before its concentration reached an equilibrium in the system. The contact time should start from that moment).

In the SPC for both preventive and curative treatments, the frequency of applications (per day or per week) should be mentioned.

Temperature:

Regarding the temperature, an average temperature ranging from 20 to 30°C should be used.

This temperature range is representative of the temperatures met in processing systems where problems can occur. This range also allows the growth of microorganisms in the laboratory.

If other temperature ranges are necessary due to the specific nature of the respective claim, a scientifically sound justification must be provided by the applicant.

5.5.11.3.5 Acceptance criteria

The required tests should be performed (using the required test organisms and test conditions), in accordance with the general test guidelines laid out in sections 5.5.1-5.5.5.

The following criteria are usually the minimum requirements that need to be demonstrated:

Preventive treatment

See section 5.5.11.3.4 for a description of the preventive action of a biocide.

Validity of the studies: Regardless of the method used, growth of the test organisms must be demonstrated in the untreated controls. Growth means an increase over the recovery directly after inoculation which is statistically significant and greater than 0.5 lg[36]. In justified cases, it can also be acceptable to show the degradation of the matrix instead. In that case, processing fluid from the field could be used.

Efficacy criteria for all the target organisms: regardless of the method used, compared to recovery directly after inoculation of the biocide-treated inoculated samples, no growth of the target organisms must occur.

Curative treatment

See section 5.5.11.3.4 for a description of the curative action of a biocide.

Validity of the study: In the inoculated vessels, growth in the untreated control does not need to be shown, nevertheless the initial size of the inoculum should be sufficiently high to show a reduction. Over the course of treatment, cell numbers in the biocide-free inoculated controls must remain stable or increase when compared to directly before treatment. A decrease of the level of inoculum in the control in this period is acceptable only if it is not statistically significant and not greater than 50%.

Efficacy criteria:

The reduction is defined by the comparison of the level of inoculum in the treated sample at t=0 to the level of inoculum in the treated sample at the end of the contact time.

- for bacteria: 3 lg reduction is the minimum level of performance;
- for yeasts and fungi: 2 lg reduction is the minimum level of performance;
- for biofilm: 2 lg reduction is the minimum level of performance.

Efficacy criteria should be achieved for the claimed contact time.

5.5.11.4 Preservation of in-use wood treatment solution

5.5.11.4.1 Introduction

For industrial wood preservation treatment, water-based solutions containing one or more decay preservatives and various wood treatment additives may be used to preserve the integrity and stability of treated wood from exposure to natural weathering during use. The liquid process preservative is used to maintain the quality of the wood treatment solution by preventing the growth of microorganisms that could accumulate in storage tanks, block pumps and/or pipework. The liquid process preservative is not intended to be a wood preservative.

In such industrial applications, a significant volume of treatment solution is prepared from a concentrate product:

- For dipping application, the wood is immersed in the treatment solution for a defined exposure time and then removed for drying. A new batch of wood is then treated with the same solution leading to new contamination of the wood treatment solution (process fluid).
- For penetrative treatment, the wood is introduced in the tank, a vacuum is applied followed by the introduction of the treatment solution and then pressure is applied. After breaking the pressure, the treatment solution is removed and stored before its re-use (leading to new contamination).

5.5.11.4.2 Purpose of the treatment

The biocidal products are used to maintain (preventive action) the population of micro-organisms (moulds, biofilm) in the aqueous wood preservative treatment solution. Wood treatment solutions are recirculated/re-used which leads to fouling from the wood, sawdust, etc. The accumulation of biofoulings in the installation (storage tanks, pumps and pipework) can prevent the proper operation of the installations.

5.5.11.4.3 Mode of application

Different types of application modes can be used: e.g. manual dosing, addition with a dosing pump, special dissolving/dosing device for solid biocides.

Preventive treatment

- Batch application: biocide(s) is/are injected regularly or at a frequency that does not ensure the maintenance of a constant efficacious concentration in the system. The concentration of the biocide is maximal after the injection and decreases to the minimum concentration with time.

5.5.11.4.4 Data requirements

The efficacy data presented in the dossier should be consistent with the microbial contamination related to the use.

For preventive action, the biocidal treatment is applied to the matrix and then the challenge consortium is added. A mixed consortium of a relatively small size is employed to both model a clean system becoming 'inoculated' through environmental contamination and to ensure that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved in the untreated specimens. The samples are incubated for a contact time appropriate for the biocide being tested and then analysed. The treatment is considered effective when growth in the matrix and the formation of biofilm on the coupons immersed in it is prevented in the treated specimens.

Test methods

To demonstrate the efficacy of the product, the efficacy test should be designed to simulate the use of the product. The product should be challenged and re-challenged with representative target organisms of this area (see Table 31), and by adding sawdust coming from the field.

It should be ensured that:

- in the untreated control, over the tested period growth is shown (for example by reducing the size of the initial inoculum to achieve growth in the control matrix);
- In the treated samples, between two inoculations, no regrowth is observed. If growth is observed, the test should be discarded.

If no guideline is available for a specific use of a product, or if guidelines are not suitable, the applicant may use other methods (such as intra-company Standard Operating Procedures), where the studies are scientifically robust, well-reported and provide a clear answer to the question. The general requirements for preservative testing laid out in sections 5.5.1-5.5.5 should be respected. In addition, the test methods used, together with the test conditions, should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with modifications to make the guideline more suitable for the specific product or use conditions, is also possible.

When standards are modified or non-standard methods are used, a scientific justification has to be presented. Furthermore, such deviations should be agreed on with the CA before doing the tests.

Test organisms

Table 31 below describes the minimum mandatory and optional target organisms for the different target sites. Every additional target organism claimed (not presented in this table) should also be justified and supported by suitable efficacy data. For the demonstration of the efficacy against bacteria, yeasts or fungi, tests performed with consortia of the strains presented in Table 31 are required. The growth of each target organism group should be assessed separately.

Test conditions

For preventive treatment, the contact time is not relevant and will not be declared in the SPC of the product. Test durations for preventive treatment should be chosen in accordance with the respective claims and with validity requirements.

For the frequent batch application of the biocidal product, the test duration should provide sufficient time for the untreated control to grow. Multiple dosing of the biocidal product is acceptable, if necessary, in order to reflect the claimed conditions of use; in such cases, water/solvent should be dosed in the controls accordingly.

In case of a low-frequency batch application, i.e. when the application interval of the biocidal product is longer than the time required to achieve growth in controls, the test duration should be as long as the claimed application interval.

In the case where field tests are provided, other contact times (according to the claims) can be used.

In case of dose ranges, efficacy testing should demonstrate efficacy at the minimum intended dose.

5.5.11.4.5 Acceptance criteria

The required tests should be performed (using the required test organisms and test conditions), in accordance with the general test guidelines laid out in sections 5.5.1-5.5.5.

For the demonstration of preventive action, laboratory tests should show that in the absence of a biocidal treatment, a microbial population can grow in the wood treatment solution whereas in the treated sample no growth is observed over the testing period.

Table 31: Preservation of processing liquids: test organisms according to the claims

Uses: Preservation of processing liquids		Bacteria			Fungi	
					Yeasts	Mould
Target site	**Purpose**	*Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Aeromonas hydrophila*, **AND** *Micrococcus luteus*	*Enterococcus faecium*	*Legionella pneumophila*	*Rhodotorula mucilaginosa* **AND** *Candida albicans*	*Cladosporium cladosporioides* **AND** *Penicillium purpurogenum*
Air washers, Air scrubbers, AHU/air conditioning units including adiabatic air cooling	Preservation of water to maintain or reduce the population of microorganisms (bacteria and if appropriate fungi)	X	O	O	O	O
Pasteurisers, Sterilisers and conveyor lubricants		X	X	O	O	O
Preservation of liquids used in closed/opened recirculating heating system and associated pipework		X	O	O	O	O
Wash waters, Transport waters		X	O	O	O	O
Target site	**Purpose**	*Pseudomonas aeruginosa*	O	O	O	*Aspergillus niger*, *Penicillium funiculosum* **AND** *Trichoderma viride*
Wood solution treatment preservation	Preservation of the wood treatment solutions during the treatment process	X				X

X: mandatory; O: optional

5.5.12 PT12 Slimicides

5.5.12.1 Introduction

This guidance describes the nature and extent of data which should be available to support the label claims for biocidal products within the main group 2 preservatives, product type 12, as described in the Annex V of the BPR for:

MAIN GROUP 2: PRESERVATIVES

Product type 12 (slimicides)
Products used for the prevention or control of slime growth on materials, equipment, and structures, used in industrial processes, e.g. on wood and paper pulp, porous sand strata in oil extraction.

Some uses are borderline between product types 11 and 12 and also with other product types. For uses for which PT allocation is not clear the relevant CA should be consulted.

For products with uses that do not fit in one of the described uses, it is up to the applicant to demonstrate efficacy in an appropriate way. In that case, a justification for the relevance of the tests used should be provided. The general guidance principles laid out in sections 5.5.1-5.5.5 of this guidance need to be respected. If needed, the test design should be discussed with and agreed upon by the CA before testing takes place. The evaluation will be done on a case-by-case basis by the CAs.

In many industrial systems, the prevention of slime formation on materials, equipment and structures used in industrial processes, is essential to maintain their function and efficiency.

In the paper industry, sessile microbial communities colonise pipework and the surfaces of materials and equipment, reducing both paper quality and flow rates of the fluids or inducing corrosion phenomena leading to structural failure and generation of foul odours.

In the oil industry, sessile microbial communities colonize the well through biofilm formation during its construction as well as the injection system/injection wellbore, reservoir, deaerators during the process of oil recovery, leading to corrosion, loss of functionality and rheological loss.

According to the PT12 definition in the BPR, the uses targeted are mainly:

- In papermaking processes, the formation of slimes and biofilms leads to losses in efficiency and can lead to a decrease of the quality of the end-product and flocculation of the pulp leading to defects caused especially by filamentous bacteria and fungi leading to breakages of the paper stream during the manufacturing process, leading to plant shut down and lost production.
- In the oilfield industry the formation of biofilms leads to the phenomenon of biologically induced corrosion, the generation of hazardous gas, or souring of the reservoir.

5.5.12.2 Paper industry

The main applications for biocides in papermaking processes are outlined below:

Prevention/control of slime growth on materials, equipment and structures of the paper machine/process

<u>The wet end:</u>

The "wet end" of the paper machine is a general term for parts of the system that involve slurry or fibres, fillers and other additives to form a wet web of fibre in a continuous fabric loop. Biocidal products are used to limit the growth of sessile bacteria which can cause slime deposit and holes in the paper.

<u>White water, clear water and cloudy water:</u>

Process water within a paper machine system especially refers to water that is drained from paper as the sheet is being formed. This is white water (definitions in Appendix 26). The water appears white in many cases due to the presence of fine fibres, fillers and air bubbles that

scatter light. This water is re-used to dilute the paper pulp. This recycling increases the amount of nutrients and metabolites resulting in an enhancement of microbial growth. Biocidal treatments are applied to control the level of microbial contamination and prevent the formation of biofilm.

Pulp (including broke, recycled pulp, machine chest) during machine shut down: e.g. in case of paper breakage or for maintenance/cleaning purposes, biocidal treatments occur on the pulp before restarting the paper machine. Pulps can be kept for days or weeks and even transported around the globe for use at other locations.

De-inking process, pulping process slurries, raw materials and chemical additives

The de-inking process is an industrial process where printing ink is removed from paper fibres of recycled paper. The result of the process is a de-inked pulp. Several steps are needed and biocidal products are applied to control the level of contamination and also to prevent the formation of biofilm.
In the pulping process, a blend of an appropriate proportion of hardwood, softwood and/or depending on the paper mill, recycled fibres is prepared, diluted and mixed with different raw materials and chemical additives to reach the expected characteristics of the final sheet of paper.
Pulp is pumped through a sequence of tanks (chest) and are kept agitated with propellers (ensure consistency of pulp slurries) or is directly brought to the paper machine without intermediate storage. Biocidal treatments are applied to prevent or control the formation of biofilm, and also to prevent the degradation of the different kinds of pulps and additives used for manufacturing the pulp before its transformation to a paper sheet.

Treatment of raw water for the papermaking process

Pulp and paper mills need large amounts of water for nearly all stages of paper production. Note that the production of one ton of paper requires 10-400 m^3 water, according to paper specialty. Therefore, paper factories often recycle their waste waters which are purified in an on-site sewage treatment plant.

The requirements for water quality vary widely in the paper industry, the quality of paper made and the production processes employed in the given pulp and paper mill. Raw waters are usually extracted from rivers and then treated with a biocidal product to ensure an acceptable level of microbial contamination.

5.5.12.2.1 Purpose of the treatment

In the papermaking process, a continuous input of microorganisms by water, fibres, high amounts of easily accessible carbon and nutriment sources (that favour strong microbial growth), air and paper additives leads to a specific microbiological equilibrium for each paper plant.

In the papermaking process biocidal treatments occur:

- In pulp and paper mills to treat the fresh water (definitions in Appendix 26) usually used as process water, which contains microorganisms that are undesired in the subsequent paper processes (deposition or production problems). The treatment type is highly related to the quality of the water source.
- In the wet-end of paper mills to treat pulp and white waters in order to control the growth of slime-producing organisms in the circulating process water system.
- In some parts of the installation of the paper mill to treat pulp and water, where conditions can lead to the production of foul odours (e.g. volatile fatty acids produced by anaerobic bacteria during fermentation of polysaccharides (starch, cellulose, etc.) or proteins; the production of hydrogen sulphide by sulfate-reducing bacteria).
- In the de-inking process during paper recycling to treat the water in order to control slime and microbial release of catalase enzymes that interfere with the process.
- On the raw materials and to the chemical additives solutions which are premixed before application, used in the papermaking process to prevent spoilage of the installation and ensure the expected quality of the end product. (e.g. biocides are added to coating slurries to prevent discolouration and agglomeration due to bacterial activity).

The objective of the biocidal treatment is often not to eliminate the microbial population but to prevent the formation of biofilms and the flocculation of the feed pulp. Depending on the level of fouling, curative treatments are also applied to reduce the biofilm already in place on materials. A biocidal product can be applied at various locations in the system depending on the purpose of the treatment.

5.5.12.2.2 Mode of application

In the paper industry, biocidal products are foreseen to be added with a dosing pump or other relevant systems. Furthermore, several dosage regimes can be applied:

Preventive treatment:

Continuous application: biocide is injected continuously or at a frequency that enables the maintenance of the concentration of biocide in a defined range.

Batch application: biocide(s) is/are injected regularly or at a frequency that does not ensure the maintenance of a constant efficacious concentration in the system. The concentration of the biocide is maximal after the injection and decreases to a minimum concentration with time.

Curative treatment:

A biocide is injected as a shock dosage at a concentration, which is usually higher than preventive treatment in response to unexpected and/or heavy microbiological growth.

Due to the specificity of each system (e.g. volume, capacity, rate of evaporation, blowdown) or due to the possible degradation/accumulation of biocides linked to the holding time index, the frequency of dosing cannot be validated beforehand. The following instruction for use should be added to the SPC:

"*It is the responsibility of the end-user to determine the effective dose on-site (e.g. by chemical or microbiological tests) for the specific location/system to ensure that the system is efficacious under the use conditions. If needed, the manufacturer of the preservative product can be consulted.*"

5.5.12.2.3 Data requirements

The efficacy data presented in the dossier should be representative of the use claimed, i.e. consistent with the kind of contamination and matrices related to the use, with the oxic conditions and with the mode of application/regimes. The level of contamination should also be consistent with the level observed in the field. Tests submitted in the dossier should be representative of the use claimed.

Preventive action

For preventive action, the biocidal treatment is applied to the matrix and then the challenge consortium is added. A mixed consortium of a relatively small size is employed to both model a clean system becoming 'inoculated' through environmental contamination and to ensure that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved in the untreated specimens. The samples are incubated for a contact time appropriate for the biocide being tested and then analysed. The treatment is considered effective when growth in the matrix and the formation of biofilm on the coupons immersed in it is prevented in the treated specimens.

Curative action

For curative action, multiple samples of a matrix are inoculated with a mixed consortium of relatively small size so that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved. Once growth has been achieved samples selected at random are either treated with the biocide or left untreated (or treated with water/the solvent used for the biocide treatment). The samples are incubated for a contact time appropriate for the biocide being tested and then analysed. The objective is that no decrease in the size of the populations should be observed in the untreated samples during this contact time, but a decrease (curative effect) should be observed in the treated samples.

Test methods

For the prevention/control of biofilm growth on materials, equipment and structures of the paper machine or process:

Preventive treatment

For bacteria, yeasts or fungi, laboratory tests should be performed according to methods such as:

- IBRG FFG19-008.2 or;
- modified ASTM E1839 where the level of inoculum is lowered and the concentration of nutrients (yeast extract) is modified in order to show growth in the untreated controls

These tests should be performed with a typical paper pulp or white water sample, extracted from the field, or with a synthetic model[37] and tested with relevant reference strains of the target organisms, as described in the methods.

For the preservation of the de-inking process, pulping process slurries, raw materials and chemical additives:

Preventive treatment

For the pulp preservation (de-inking process), for bacteria, yeasts or fungi, laboratory tests should be performed according to methods such as:

- IBRG FFG19-008.2 or;
- modified ASTM E1839 where the level of inoculum is lowered and the concentration of nutrients (yeast extract) is modified in order to show growth in the untreated controls.

The test should be performed with a typical de-inking paper pulp, extracted from the field and tested with relevant reference strains of the target organisms, as described in the methods or with a synthetic model[38].

For the preservation of aqueous raw materials used in the paper industry when the respective use is assigned to PT 12:

- for bacteria, laboratory tests should be performed according to a method such as ASTM E723, where the level of inoculum is lowered and the concentration of nutrients (yeast extract) is modified in order to show growth in the untreated controls;
- for yeasts or fungi, laboratory tests should be performed according to a method such as ASTM E875.
- alternatively, a laboratory test performed according to IBRG PDG16-007 may be used for bacteria, yeasts or fungi.

The test should be performed with a typical aqueous raw material extracted from the field and tested with relevant reference strains of the target organisms.

Note: it is not possible to define a synthetic matrix for the following matrices: pigment slurries, adhesives, dye rosin, polymer, sizing solutions and other materials. For these matrices, a test should be conducted with a representative sample of the matrix tested at its use concentration in the process.

For the treatment of raw water for the papermaking process:

Preventive treatment

For bacteria, yeasts or fungi, laboratory tests should be performed according to methods such as:

- IBRG FFG19-006.2 or;
- modified ASTM E645 where the level of inoculum is lowered and the concentration of nutrients (yeast extract) is modified in order to show growth in the untreated controls.

This test should be performed with typical raw water extracted from the field or with synthetic model water[39] tested with relevant reference strains of the target.

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[37] Examples of reconstituted pulp are provided in the ASTM E1839-20 and in the IBRG FFG19-008.2

[38] A reconstituted deinking pulp can be based on ASTM E1839-20 (or IBRG FFG19-008.2) with the addition of anionic surfactant in order to simulate the residual surfactant obtained from the deinking process.

Curative treatment

For bacteria, yeasts or fungi, laboratory tests should be performed according to a method such as ASTM E1839 using raw water extracted from the field or with a synthetic model water[39] tested with relevant reference strains of the target organisms.

If no guideline is available for a specific use of a product, or if guidelines are not suitable, the applicant may use other methods (such as intra-company Standard Operating Procedures), where the studies are scientifically robust, well-reported and provide a clear answer to the question. The general requirements for preservative testing laid out in sections 5.5.1-5.5.5 should be respected. In addition, the test methods used, together with the test conditions, should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with modifications to make the guideline more suitable for the specific product or use conditions, is also possible.

When standards are modified or non-standard methods are used, a scientific justification has to be presented. Furthermore, such deviations should be agreed on with the MS CA before doing the tests. In all cases, a scientifically robust test protocol should be agreed on with the eCA before testing.

Test organisms

Table 32 below describes the minimum mandatory and optional target organisms for the different target sites. Every additional target organism (not presented in this table), should also be justified and supported by suitable efficacy data.

For the demonstration of the efficacy against bacteria, yeasts and fungi, tests performed with consortia of the strains presented in Table 32 are required. The growth of each target organism group should be assessed separately.

Test conditions

Conditions should be representative of the uses claimed (oxic/anoxic). Note that for *Desulfovibrio* sp. anoxic condition in the test is required.

In case of dose ranges, efficacy testing should demonstrate efficacy at the minimum intended dose.

Contact time

For preventive treatment, the contact time is not relevant and will not be declared in the SPC of the product. Test durations for preventive treatment should be chosen in accordance with the respective claims and with validity requirements.

For the continuous or frequent batch application of the biocidal product, the test duration should provide sufficient time for the untreated control to grow. Multiple dosing or continuous dosing of the biocidal product is acceptable if necessary in order to reflect the claimed conditions of use; in such cases, water/solvent should be dosed in the controls accordingly. In case of a low-frequency batch application, i.e. when the application interval of the biocidal product is longer than the time required to achieve growth in controls, the test duration should be as long as the claimed application interval.

For curative treatment, information on contact times should be included in the SPC. The duration of curative efficacy testing should reflect the label claims.

In laboratory tests, for batch application, normally these contact times should be applied:

- for a fast-acting product an exposure time ranged from 1 to 3 hours;
- for a slow-acting product an exposure time ranged from 6 to 24 hours.

In the SPC for both preventive and curative treatments, the frequency of applications (per day or per week) should be mentioned.

[39] Example of reconstituted raw water: the array of water sources that can be used for paper mill make-up water is very large. Sources can vary from ground water – river/lake/ reservoir – to borehole water. The chemistry of each is very different. An example of synthetic model water has a total hardness of 30 to 300 mg/l $CaCO_3$, conductivity of 100 to 600 µS/cm at 25°C and a pH range from 6 to 9.

Temperature:

Regarding the temperature to be used, the source of the water is most of the time surface water which temperatures are ranged from 0 to 30°C. Nevertheless, an average temperature range from 20 to 30°C should be used.

If other temperature ranges are necessary due to the specific nature of the respective claim, a scientifically sound justification must be provided by the applicant.

5.5.12.2.4 Acceptance criteria

The required tests should be performed (using the required test organisms and test conditions), in accordance with the general test guidelines laid out in sections 5.5.1-5.5.5 of this guidance. The following criteria are usually the minimal requirements that need to be demonstrated.

Preventive treatment, regardless of the use considered:

Validity of the studies: regardless of the method used, growth of the test organisms should be demonstrated in the untreated control. Growth means an increase over the recovery directly after inoculation which is statistically significant and greater than 0.5 lg[36].

In justified cases, it can also be acceptable to show the degradation of the matrix instead.

Efficacy criteria for all the target organisms: regardless of the method used, compared to recovery directly after inoculation of the biocide-treated inoculated samples, no growth of the target organisms must occur.

Curative treatment

Validity of the study:

In the inoculated vessels, growth in the untreated control does not need to be shown, the initial size of the inoculum should be sufficiently high to show a reduction. Over the course of treatment, cell numbers in the biocide-free inoculated controls must remain stable or increase when compared to directly before treatment. A decrease of the level of inoculum in the control in this period is acceptable only if it is not statistically significant and not greater than 50%.

Efficacy criteria:

The reduction is defined by the comparison of the level of inoculum in the treated sample at t=0 to the level of inoculum in the treated sample at the end of the contact time.

- for bacteria: 3 lg reduction is the minimum level of performance.
- for yeasts or fungi: 2 lg reduction is the minimum level of performance.

Table 32: Papermaking process: test organisms according to the claims

Uses: Papermaking process		**Bacteria**			**Fungi**	
					Yeasts	**Mould**
Target site	**Purpose**	*Pseudomonas aeruginosa, Enterobacter cloacae* **AND** *Aeromononas hydrophila*	*Pseudomonas aeruginosa* **AND** *Klebsiella aerogenes* (formerly *Enterobacter aerogenes)*	*Desulfovibrio* sp.	*Rhodotorula mucilaginosa* (formerly *R. rubra*) **AND** *Candida albicans*	*Chaetonium globosum* **AND** *Aspergillus niger*
Wet end/white water, pulp (including during machine shut down)**, thick stock** (including broke, blend, machine chest as well as the recycled pulp)**. White water, cloudy water and clear water. Thin stock**	Prevention or control of slime growth on materials, equipment and structures of a paper machine or process	O	X	O	O	O
De-inking process and pulping process, slurries, raw materials and chemical additives (e.g. starch preparations)**, wet end additives**	Prevention of growth of bacteria, and/or yeasts and/or moulds in water-based formulations used in the paper production	O	X	O	O	O
Treatment of raw water for papermaking process	Treatment of fresh water: maintain or reduce the population of microorganisms to an acceptable level (bacteria and if appropriate fungi)	X	O	O	O	O

X: mandatory; O: optional

5.5.12.3 Oil industry

During the construction of the well, drilling fluids are used to aid the drilling of boreholes into the earth and also to protect the drill. Biocides are applied to drilling fluids to prevent loss of functional performance (e. g. rheological properties and fluid loss) and to prevent microbial contamination of the well through biofilm formation. Many oilfields have soured due to the contamination of wellbores with sulfate-reducing bacteria (SRB) and thiosulfate-reducing bacteria (TRB, whose metabolites include H_2S which is responsible for souring).

When the well is in place, during the secondary processes of oil recovery, injection fluids e.g. water is pumped down. Biocides are used to:

- prevent microbiologically influenced corrosion (MICor) in the injection system;
- prevent biofouling of the injection system/injection wellbore;
- prevent/reduce microbial contamination and biofilm formation in the reservoir by reducing the number of viable bacteria injected into the reservoir;
- prevent/reduce biofilm formation in the deaerators.

When submitting a dossier, particular attention should be paid to the description of the intended use in particular on the purpose of the treatment.

Treatment programmes are optimised to achieve a steady state, whereby the growth of micro-organisms is balanced by killing and physical removal (where possible) of the micro-organisms. This is specifically applied in pipelines, or systems that can be pigged (pigging is the practice of sending a close-fitting device down a pipeline for maintenance or monitoring operations and is done without stopping the flow in the line). In essence, the objective is to maintain an acceptable number of sessile bacteria over a defined time period, by optimising the frequency duration of dosing and concentration of the biocide, coupled with regular pigging/cleaning.

5.5.12.3.1 Introduction

The main oilfield applications for biocides are outlined below:

Preservation of drilling fluids (including drilling muds)

In geotechnical engineering, drilling fluids are used to aid the drilling of boreholes into the earth. The drilling fluids are also used for suspending and carrying away drill cuttings from the drill head.

Among the drilling fluids, drilling muds are used to provide hydrostatic pressure that prevents fluids (e.g. water) from entering the well, keeping the drill cool and clean during the drilling process and preventing corrosion. Drilling fluids or "muds" are usually applied in a circulating system. The fluid flows through the drilling head and is pumped back to the surface. After the removal of stones etc., the fluid is often re-used. Biocides are added to prevent microbial deterioration and therefore loss of functionality of the drilling fluids.

Thus, biocidal products are used to treat drilling fluids/muds to prevent the development of bacteria capable of forming biofilms aggravating the corrosion of the metal pipes or cements necessary for sealing the wells. Biocides are used to prevent rheological loss and fluid loss control. They are also used to prevent microbial contamination of the wellbore.

Pipelines and hydro-testing

Pipelines (e.g. inter-platform pipelines) may be used for transporting seawater, produced water for discharge or injection, total fluids, or export fluids (hydrocarbons) or oil. In each case, there is likely to be a need to treat the matrix with biocides to prevent and control microorganisms.

A hydrostatic test (or leaking test) is a technique for checking for the strength or possible presence of leaks in pressure vessels (pipelines, boilers, vessels, gas cylinders, fuel tanks, etc). The vessel or the pipe system to be tested is filled with a liquid (usually water) often dyed and pressurised to the specified pressure. Hydro-testing may also be employed during mothballing of pipelines.

In case of a hydrostatic test or leaking test, fluids used are typically left at least for several days and sometimes more. Therefore, the device tested can be subject to corrosion induced by bacterial growth (then biofilm formation) over time if microorganisms inducing corrosion are not controlled. To prevent this phenomenon, corrosion inhibitors, oxygen scavengers and biocides may be used.

The main difference between normal use in pipelines and hydrostatic tests is that normal pipeline operation involves constant flow, while a hydrostatic test consists of a static shut-in.

Water injection systems

For enhancing the recovery of oil, water is injected into oilfields to increase the pressure in the reservoir. The water injected is treated by biocides to prevent biofouling, microbially induced deterioration and corrosion in equipment upstream of injection wellhead. The water injection may also be used to transfer biocide into the oil reservoir to prevent the growth of some bacteria which can promote the corrosion phenomena and the generation of hazardous gas (hydrogen sulfide).

Fracturing[40]

Hydraulic fracturing can involve the pumping of synthetic polymers, such as hydrolysed poly acrylamide modified (HPAM), at high pressure into the reservoir to open fractions by water pressure. This produces a complex matrix of microfractures. The HPAM is typically chemically modified and either anionic or cationic in nature. Alternatively, a "gel-frac" approach may be used where a biopolymer such as a guar is pumped into the reservoir with a proppant to induce fractures via viscosity and place the proppants to keep the large fractures open. Biocides are used in fracturing to pre-treat the water.

Whilst biocide may be applied to protect polymers during pre-use storage (PT6), the intention is to protect the formation, and also to control bacteria in the fracturing fluid, and prevent growth while the fracturing fluids are "shut-in".

Please note that national legislation in some member states may contain further restrictions on hydraulic fracturing uses, which are not specifically addressed in this guidance.

5.5.12.3.2 Mode of application

In the oil and gas industry, even a new well will have existing microbial loads, and during interventions, or during the pumping of (sea) water into the well, additional microbial communities may be introduced. The intention of PT12 applications will be to treat existing and new microbial contamination, to achieve acceptable background levels.

Different types of applications can be used (e.g. manual dosing, addition with a dosing pump, special dissolving/dosing device for solid biocides, or generator for in-situ production of biocide substance).

Continuous application: biocide is injected continuously or at a frequency that enables the maintenance of the concentration of biocide within a defined range.

Batch application: biocide(s) is/are injected regularly or at a frequency that does not ensure the maintenance of a constant efficacious concentration in the system.

Due to the specificity of each system to be treated, the frequency of dosing cannot be validated beforehand. The user of the product should check on site (e.g. by microbiological tests, on the measurement of microbial activity or by measuring the by-products of bacterial activity, e.g. H_2S formation) in order to determine the dosing program (efficient dose and frequency) for the specific location/system. This statement should be added to the SPC.

5.5.12.3.3 Data requirements

The efficacy data presented in the dossier should be representative of the uses claimed, i.e. consistent with the microbial contamination related to the use and with the mode of application. The efficacy of the minimum intended dose needs to be demonstrated by the submitted efficacy data.

Curative action

For curative action, multiple samples of a matrix are inoculated with a mixed consortium of relatively small size so that the growth of the consortium in the matrix and the development of a biofilm on the coupons submerged in it can be achieved. Once growth has been achieved samples selected at random are either treated with the biocide or left untreated (or treated with water/the solvent used for the biocide treatment). The samples are incubated for a

[40] An overview of hydraulic fracturing and other formation stimulation technologies for shale gas production. https://publications.jrc.ec.europa.eu/repository/handle/JRC86065.

contact time appropriate for the biocide being tested and then analysed. The objective is that no decrease in the size of the populations should be observed in the untreated samples during this contact time, but a decrease (curative effect) should be observed in the treated samples.

The efficacy of a biocide in any given oilfield application will depend on various factors e.g.:

i. use concentration;
ii. contact time with target microorganisms;
iii. the combination of ambient temperature and pressure;
iv. the pH;
v. the presence of other chemicals.

The latter factors (iii-v) are highly variable between sites, wells and applications, they also have varying effects on the efficacy complicating results. Therefore the end-user should check on-site (e.g. by chemical or microbiological tests) in order to determine the effective dose for the specific location/system.

The following instruction for use should be added to the SPC:

"It is the responsibility of the end-user to determine the effective dose on-site (e.g. by chemical or microbiological tests) for the specific location/system to ensure that the system is efficacious under the use conditions. If needed, the manufacturer of the preservative product can be consulted."

The presence of other chemicals is both likely and another complicating factor. These may be naturally occurring or formation chemicals, or added chemicals used as process aids. Biocides are generally reactive and can be deactivated through reactions with incompatible additives or other chemicals, reducing or eliminating biocidal efficacy. In oilfield applications, reducing agents such as some antioxidants and oxygen scavengers will neutralise a wide range of biocidal active substances. The impact a biocide has on a scale and corrosion inhibitor films is sometimes overlooked. It is generally accepted that most biocides will deactivate oxygen scavengers, and these are dosed as far apart as possible. For fracturing applications, determining biocidal efficacy as a function of Total Dissolved Solids (TDS) should be considered as this can have an effect. Another example is natural chemical interference, such as wells with high calcium levels which adversely impact the efficacy of quaternary ammonium-based biocides. The applicant should adequately address these issues in their dossier, in order to cover them in section 3.5.7 Known limitations of the PAR.

Therefore, the test methods in the following section will consider efficacy against known target organisms, establishing a minimum biocidal concentration.

Test methods

In the oil industry, only efficacy against bacteria (see Table 33) is expected.

For planktonic bacteria, laboratory tests should be performed according to a method such as:

- modified method according to general principles of the IBRG FFG21-011.1

The objective of this efficacy test is to demonstrate that a given product is able to reduce the level of contamination on an inoculum representative of the field with a representative physiological state.

Therefore the method chosen should be adapted, in order to show growth in the first step (ensure that the bacteria are under a representative physiological state), followed by the second step where the product shows its ability to reduce the level of contamination reached at the first step according to the efficacy criteria (see section Acceptance criteria):

1. the level of inoculum is adjusted in order to facilitate growth in the control samples.
2. when the level of contamination is sufficient[41] and reached a level that allows the demonstration of the efficacy (see section Acceptance criteria), the biocidal product is added to the treated samples at the claimed concentration. Efficacy is then assessed after the contact time. In the untreated control samples, the level of contamination should remain stable or increase during the contact time.

[41] Growth means an increase over the recovery directly after inoculation which is statistically significant and greater than 0.5 lg (Student test with a p-value <0.05 (95 % confidence level) recommended, p <0.1 (90% confidence level), if justified (e.g. identifying outliners, or lowest concentration with a biocide showing growth)

The test should be performed either with typical matrices extracted from the field or with a relevant, defined synthetic matrix (see available proposal for synthetic water[42]). The composition of the synthetic matrix should be carefully justified. Defined strains of the target organisms should be used for testing. Both untreated control and treated samples should be performed at least in triplicates.

For biofilm:

For sessile bacteria, laboratory simulated-use efficacy tests should demonstrate the ability of the product to exert a controlling effect on the biofilm under either static conditions or flow conditions depending on the use pattern (claim). This trial should be performed according to a method such as NACE TM0194-2014, on mature biofilm samples taken from the field[43] or according to a modified method such as IBRG FFG21-010.1 and IBRG FFG21-011.1 in two steps:

1. samples of the model matrix containing the sampling coupons are inoculated with a consortium of bacteria and cultured under representative conditions.
2. when the biofilm is established on the coupons, the biocidal product is added at the application rate into the treated samples. Efficacy is then assessed after the contact time, according to the efficacy criteria of section Acceptance criteria. In the untreated control samples, the contamination should remain stable or increase during the contact time.

Both untreated control and treated samples should be performed at least in triplicates.

If no guideline is available for the specific use of a product, or if guidelines are not suitable, the applicant may use other methods (such as intra-company Standard Operating Procedures), where the studies are scientifically robust, well-reported and provide a clear answer to the question. The general requirements for preservative testing laid out in sections 5.5.1-5.5.5 should be respected. In addition, the test methods used, together with the test conditions, should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with modifications to make the guideline more suitable for the specific product or use conditions, is also possible.

When standards are modified or non-standard methods are used, a scientific justification has to be presented. Furthermore, such deviations should be agreed on with the evaluating Competent Authority before doing the tests. In all cases, a scientifically robust test protocol should be agreed upon on with the CA before testing.

Test organisms

Table 33 describes the minimum mandatory and optional target organisms for the different target sites. For each group of bacteria (GHB, SRB, TRB and APB), two strains that represent different genera should be tested. Note that for TRB and APB, some strains (respectively Methanogen (*Archaea*) and *Clostridium formicaceticum*) should be obligatorily tested.

Every additional target organism claimed (not presented in this table) should also be supported by suitable efficacy data.

For the demonstration of the efficacy against the different functional groups of bacteria, tests performed with consortia of the strains presented in Table 33 are required. The growth of each functional target organism group should be assessed separately.

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[42] Standard synthetic formation water: It should be recognised that formation waters are highly variable (Thyne, Geoffrey, and Brady, Patrick. Evaluation of formation water chemistry and scale prediction: Bakken Shale. United States: N. p., 2016. Web. https://doi.org/10.1016/j.apgeochem.2016.10.015), the synthetic formation water matrix should have the following specifications:
10^2 – 10^5 mg/l K^++ Na^+, Cl^-
10^2 – 10^4 mg/l Ca^{2+}, Mg^{2+}, SO_4^{2-}, CO_3^{2-}, HCO_3^-
Other salts or scale may be required if relevant to the particular location, application or particular formation water or microbial environment and should be justified accordingly. Other examples of synthetic matrix is presented in the IBRG FFG21-010.1, Annex VIII

[43] Recommendations are presented in the NACE TM0194-2014.

Test conditions

In case of dose ranges, efficacy testing should demonstrate efficacy at the minimum intended dose. The oxic conditions (oxic or anoxic) should be defined and should be representative of the conditions defined in the use claims.

Contact time: For curative treatment, information on contact times should be included in the SPC. The duration of curative efficacy testing should reflect the label claims. In laboratory tests, the following contact times should be applied:

- for a fast-acting product an exposure time ranged from 1 to 3 hours;
- for a slow-acting product an exposure time ranged from 6 to 24 hours.

In the SPC, the frequency of applications recommended (e.g. per day or per week) should be mentioned.

Temperature: Temperature will depend on the well, the depth and other factors. Most bacterial problems occur in the temperature range of 5 to 80°C. Above 80°C, most bacteria, even thermophiles, are very inactive. Many systems operate below 60°C.

Regarding the efficacy trial, an average temperature range from 20 to 35°C should be used as standard. An additional temperature of 60°C or e.g. 4 to 5°C for specific applications (e.g. pipeline application) may be used.

pH: The effect of pH will depend upon the biocide being used, with some having a narrow range of effectiveness. Modification of pH can also be used to deactivate residual biocide left in the process fluids and returned to the installation. The water in most systems will be between pH 6-8.5, and tests for these applications should be in this range. However, the pH within the biofilm can be lower than this due to an accumulation of acidic by-products. For this reason, sessile testing should be performed on "mature" biofilms grown under representative conditions that have had time to establish a viable population and an accumulation of metabolic by-products to make the testing more representative.

5.5.12.3.4 Acceptance criteria

Efficacy criteria:

The reduction is defined by the comparison of the level of inoculum in the treated sample at t=0 to the level of inoculum in the treated sample at the end of the contact time.

- for bacteria: 3 lg reduction is the minimum level of performance.
- for biofilm of bacteria: 2 lg reduction is the minimum level of performance.

Table 33: Oil industry: test organisms according to the claims

Target site	**Purpose**	*Pseudomonas* (*aeruginosa or fluorescens*) **OR** *Shewanella* (*oneidensis* or *putrefaciens*) **OR** *Klebsiella sp./ Enterobacter sp.*	*Desulfovibrio* sp. **AND** *Desulfotomaculum* sp.	Methanogen (*Archaea*) **AND** *Thermovirga, Anaerobaculum, Haloanaerobium* **OR** *Thermotoga*	*Clostridium formicaceticum* **AND** *Pseudomonas (aeruginosa or fluorescens)* **OR** *Shewanella (oneidensis or putrefaciens)*
Processing systems in oil extraction: Pipelines and Hydro-testing	Control of MICor via control of biofilms	X	X	X	-
Processing systems in oil extraction: Water injection systems	Control of MICor and biofouling of deaerators, filters and injection wellbore. Control of reservoir souring via control of biofilms in near injection wellbore.	X	X	X	-
Processing systems in oil extraction: Fracturing	Control of post fracturing souring, via control of biofilm within fractures.	X	X	X	-
Offshore and onshore installations: Injection fluids, produced water, etc.	Control of MICor and souring via the prevention or mitigation of biofilm build-up in water injection/ reinjection, production or distribution systems and within the oilfield reservoir or hydrostats.	X	X	X	O
Offshore and onshore installations: Preservation of drilling fluids (including drilling muds)	Control of slime and other deposits (biofilms) on inner surfaces in the production line and the drilling hole	X	X	X	O

X: Mandatory; - : not relevant; O: optional

5.5.13 PT13 Working or cutting fluid preservatives

PT13 deals with preservatives for metal working fluids during their use in industrial processes. The general principles for evaluating PT13 products can be found in section 5.5.2 to 5.5.5. IBRG[44] developed a method that allows to test the efficacy of active substances in a model matrix ("A Method for Determining the Basic Efficacy of Biocidal Active Substances used in Aqueous-Based Metal Working Fluids for their Protection in Use, IBRG FFG16-001. This method should be used, unless it is justified that the method is not relevant for this specific product.

[44] International biodeterioration research group (IBRG): www.ibrg.org

5.6 Pest Control (Main group 3)

5.6.1 General

The text for this section is under development and will be added at a future update.

Humaneness
According to the BPR (Article 19(1)(b) criterion ii and common principles point 49 and 76 in Annex VI) biocidal products should cause no unacceptable effects on the target organisms, including unnecessary suffering and pain for vertebrates (humaneness). This criterion is relevant for biocides in the Pest Control PTs 14, 15, 17, 19 (repelling or attracting vertebrates) and PT20.

For these biocides an assessment must be made to demonstrate that the biocidal product does not cause unnecessary suffering in its effect on target vertebrates. This must include an evaluation of the mechanism by which the effect is obtained and the observed effects on the behaviour and health of the target vertebrates; where the intended effect is to kill the target vertebrate, the time necessary to obtain the death of the target vertebrate and the conditions under which death occurs must be evaluated.

A biocidal product intended to control vertebrates must not normally be regarded as satisfying criterion (ii) under point (b) of Article 19(1) unless:

- death is synchronous with the extinction of consciousness, or
- death occurs immediately, or
- vital functions are reduced gradually without signs of obvious suffering.

For repellent products, the intended effect must be obtained without unnecessary suffering and pain for the target vertebrate.

Guidance on the assessment of humaneness is currently not included in Volume II Efficacy Part B/C: Efficacy Assessment and Evaluation, but some general guidance can be found in the TNsG on Product Evaluation Chapter 6.

5.6.2 PT14 Rodenticides

General introduction

This section provides guidance on the methodology for the evaluation of the efficacy of rodenticide biocidal products according to the common principles laid down in Annex VI of the BPR in order to demonstrate that the condition for granting an authorisation in Article 19(1)(b)(1) of the BPR is fulfilled (i.e. the rodenticide is sufficiently effective).

5.6.2.1 Introduction

Depending on its intended purpose, a rodenticide may be regulated as a biocidal product or as a plant protection product[45]. This document covers the rodenticides under the BPR, which are used predominantly for the control of the house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*) and the roof rat (*Rattus rattus*). Also other target species such as water voles (*Arvicola amphibius*), bank vole (*Myodes glareolus*), common voles (*Microtus arvalis*), field or wood mice (*Apodemus* spp.) and the grey squirrel (*Sciurus carolinensis*) are considered.

The four standard fields of use are given below with examples of possible fields of use:

- in and around buildings
    - in and around residential homes and other places in which people are accommodated;

[45] Biocidal product (PT14): Rodenticides used for the control of mice, rats or other rodents (by means other than repulsion or attraction) outside plant growing areas, for example in farms, cities, industrial premises etc, and inside plant growing areas not to protect plant or plant products.

Plant protection product: Rodenticides applied in plant growing areas (agricultural field, greenhouse, forest) to protect plants or plant products temporarily stored in the plant growing areas in the open without using storage facilities.

Where a product is used in both situations (as PPP and BP), it will need dual authorisation for the relevant use in accordance with the last subparagraph of Article 2(2) of the BPR. See also http://ec.europa.eu/food/plant/protection/evaluation/borderline_en.htm

    - in and around rooms intended for the preparation, processing or storage of food and beverages;
    - in and around stores, ships' holds, factories and silos;
- at waste dumps;
- in sewers
    - in moist/wet environments such as sewers and watersides;
- open areas
    - open areas such as airports or leisure areas.
    - on animal husbandry farms (pigs, poultry, cattle, etc.);

Since the majority of rodenticides are bait products, most of this guidance deals with the evaluation of the efficacy of baits. In the text it is indicated where it specifically concerns bait products or concerns other types of rodenticides.

5.6.2.1.1 Aim

The aim of this document is to provide guidance on how to assess the efficacy of rodenticides, in order to ensure that only sufficiently effective products are authorised and therefore placed on the market for use. Animal welfare considerations are also taken into account.

5.6.2.1.2 Global structure of the assessment

Full assessment of efficacy is conducted on applications for product authorisations.

Information on effectiveness and intended use(s) of the product, together with its active substance(s), must be sufficient to permit an evaluation of the product and to define its conditions of use.

Efficacy studies (see section 2 below for the type of testing required) should be performed with the product to evaluate whether the product is effective for the intended use(s) at the specified doses. Efficacy tests should be performed with the product (in its final formulation) for which the authorisation is sought, and the composition of the test-product should be provided in the efficacy reports (especially for field trials and palatability tests). Any efficacy data from scientific literature are considered only as supportive data and should not replace efficacy data obtained from efficacy tests, which should be performed according to recognised standards. Data on the mortality and, in case of bait products palatability of the bait, resulting from these studies are compared with the specified criteria. The basis for the evaluation is the uses specified in the application (i.e. draft SPC) submitted by the applicant.

5.6.2.2 Dossier Requirements

Data on efficacy are required for every application for authorisation. The following information on effectiveness is required for each biocidal product in accordance with Annex III of the BPR:

1. Function (e.g. rodenticide) and mode of control (e.g. killing);
2. Representative organism(s) to be controlled and products, organisms or objects to be protected;
3. Effects on representative target organisms;
4. Intended concentration at which the active substance will be used and application rate;
5. Mode of action (including time delay);
6. The intended uses for the product;
7. Efficacy data to support these intended uses, including any available standard protocols, laboratory tests or field trials used including performance standards where appropriate and relevant;
8. Any known limitations on efficacy:
    8.1. Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies;
    8.2. Observations on undesirable or unintended side effects for example, on beneficial and other non-target organisms.

Efficacy testing

It should be noted that any efficacy testing conducted in the European Union on rodents should be in accordance with the principles set under Directive 2010/63/EU[46] on the protection of animals used for scientific purposes. However, field trials with rodenticide products to control wild rodent infestations under actual use conditions that are carried out to demonstrate the results of already obtained data on palatability, mortality and humaneness are not considered animal procedures for the purposes of Directive 2010/63/EU.

For all types of rodenticides, efficacy has to be demonstrated in a laboratory trial and a field trial or alternatively in a semi-field trial and a field trial for each target organism submitted in the application, unless specified otherwise in this guidance. For roof rats it is also acceptable to demonstrate efficacy:

- in two or more well-conducted semi-field trials (for description see section 2.6 below), since in some regions infestations of roof rats are quite rare; or
- Two (or more) well-conducted field trial(s) in regions with infestations of roof rats.

In general it applies that tests should be of high quality to be considered for evaluation. For animal welfare reasons, in laboratory tests, the number of animals per test should be restricted to a minimum.

Positive results in field trials may outweigh negative results[47] in laboratory studies, but only under the following conditions:

- there is at least one other laboratory study (or semi-field trial) with positive results for each study with negative results and;
- there is at least one field trial of high quality with positive results.

Positive results in laboratory studies cannot outweigh negative results in field and semi-field trials.

In case of testing only in semi-field or field trials (roof rats):

- at least two well-conducted semi-field tests or one field trial should have positive results, respectively.

The following guidance is designed to be flexible and does not specify rigid protocols to which tests must be conducted. Published or unpublished data from any source will be considered provided the data are scientifically valid and relevant to the application. In all cases, the methods have to be described in sufficient detail to make the data reproducible. Ideally, data should be generated using national or internationally recognised testing methods and in accordance with the principles set under Directive 2010/63/EU on the protection of animals used for scientific purposes. However, applicants can also submit data generated using their own testing strategies where these are conducted and well reported to a sound scientific standard. In all cases, the data must allow a specific assessment of efficacy and, in case of bait products, palatability of the product. Anecdotal evidence will not be acceptable.

Assessment will be made in relation to the effectiveness of the product for the intended uses in the draft SPC submitted with the application. This assessment will take into account the animals that are considered to be harmful and are to be controlled (target species), indoor or outdoor use, the method(s) of application, application rates, use patterns of the product, maximum storage period (shelf life) of the product, together with any other specific terms and conditions concerning the use of the product.

The target species selected for efficacy testing should be appropriate to the geographic regions in which the product will be used. They should be named in the draft SPC for the product (either common or generic names may be used). Please note that in some countries specific rodent species are protected and no control action against them is permitted.

Intended uses

Examples of intended uses given in the draft SPC associated with the target organisms are :

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[46] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

[47] Negative results are those showing insufficient efficacy against the evaluation criteria (see section 4.1 of this Guidance).

- for use against house mice:
  - this will require testing against *Mus musculus*.
- for use against rats
  - this will require testing against *Rattus norvegicus* and *Rattus*.
- for use against brown rats
  - this will require testing against *Rattus norvegicus*.
- for use against rats and house mice
  - this will require testing against *Rattus norvegicus*, *Rattus* and *Mus musculus*.
- for use against rats in sewers
  - this will require testing against *Rattus norvegicus* with specifically treated bait (see section 2.4 below)
- for use against voles
  - this will require testing against at least two vole species which differ in size and behaviour, for example, water voles (*Arvicola amphibius*), bank vole (*Myodes glareolus*) and common voles *Microtus arvalis*.
- for use against a field mice (wood mice) species
  - this will require testing against the specified target species, for example the long-tailed field mouse/wood mouse (*Apodemus sylvaticus*) or yellow-necked field mouse (*Apodemus flavicollis*).
- for use against [name of target species]
  - this will require testing against the given target species. an example could be the grey squirrel (*Sciurus carolinensis*).

General intended uses given in the draft SPC, such as 'for use as a rodenticide' or 'for use against mice', with no further clarification of the target species are not acceptable. This is because it would allow use against rodent species for which the product is not tested and/or not intended. Concerning the target species, intended uses have to be species-specific (both for products authorised for professional and non-professional users).

Testing has to be species-specific, and for each target organism that is given in the draft SPC, a study should be conducted. This is because the biology, behaviour and susceptibility of target species, even within taxonomic groups such as rats, voles or mice, may differ considerably. For example, the brown rat (*R. norvegicus*) is more sensitive for anticoagulants than the roof rat (*R. rattus*), whereas it has been observed that the roof rat is more neophobic and will be less likely to accept baits than the brown rat. Mice are taxonomically very unspecific and may be applied to a broad range of species (e.g. *Mus musculus*, or various *Apodemus* species) with different biology, behaviour and susceptibility against the active substances. Vole species differ considerably in their size and habitat. Therefore, all target organisms given in the draft SPC have to be tested. If the authorisation of a rodenticide with a less specific intended use, such as 'for use against voles' or 'for use against mice' is applied for, the product has to be tested at least against all representative species of the respective taxonomic group. For voles there are products authorised under the plant protection products (PPP) legislation, but under some circumstances, there can be a need for biocidal product approvals (e.g. in case of invasions near buildings and disease spreading).

Resistance claims are allowed for products based on actives with a mode of action other than anticoagulants. For products based on anticoagulants there is differing opinions of permitting claims by Member States[48] and therefore, until further discussions and decisions are made, such intended resistance claims must be considered on a case by case basis in discussion with the Member States. An intended use such as 'for use against rats and/or mice resistant to the first generation anticoagulants', is generally not possible, because test animals which are resistant to first generation anticoagulants are difficult to define and their degree of susceptibility may vary. Moreover, when a case of resistance is recognised in a field situation, it is generally advisable to use non-chemical methods like mechanical or electronic traps, rodenticide with non-anticoagulant mode of action, or the most potent anticoagulant

[48] This issue is under review and discussion and the guidance will be updated if the situation regarding resistance claims for anticoagulants changes.

rodenticides, and the use instructions in the draft SPC should generally contain a paragraph about resistance management. Therefore, a general intended use concerning resistance on an anticoagulant product may not be regarded as informative, since resistance generally refers to the active substance rather than a specific product.

5.6.2.2.1 Test animals

Although laboratory testing should preferably be performed on second generation wild animals housed in groups, the difficulty and constraints associated with obtaining and maintaining them for testing purposes is recognised. Therefore for tests conducted within the laboratory, animals sourced from recognised commercially available strains are acceptable.

In accordance with Directive 2010/63/EU, Articles 7 and 9 and Section A, 3.2. of Annex III, , semi-field trials should preferably be conducted using wild rodents or their offspring. Although not preferred, it is possible to use strains that resemble wild strains in semi-field trials as an alternative. These strains should be outbred strains (e.g. Long Evans or Lister Hooded rats) which retain the behavioural characteristics of wild rodents, which includes neophobia, anxiety, and fully capable sensory organs (no impairment of seeing, hearing, smelling or taste). When laboratory strains that resemble wild strains are used, a short description of the behavioural characteristics as well as reasoning for the choice of the respective strain as test animals should be provided. Generally, the diet which rodents (laboratory and wild strain) receive prior to the tests can be crucial for their behaviour towards bait products. It is therefore important that, as far as possible, the study reports should also include information on the dietary history of the test animals. It is recommended that test animals should receive a rather broad diet during breeding. Where wild animals are used in laboratory or semi-field studies, these may be live trapped from the wild, reared in either outdoor colonies or under laboratory conditions such that it permits the animals to retain much of their natural physiological and behavioural characteristics. Breeding stock used for rearing wild rodents should not be selected for docile qualities or other characteristics that significantly alter their wild tendencies.

OECD Guidance Document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (OECD, 2002) must be considered. Unnecessary suffering must be avoided (e.g. excessive weight loss/severe dehydration, persistent convulsions, cannibalism/self-mutilation, etc.) and animals should be checked regularly. Moribund animals should be euthanized in line with the requirements to apply humane end-points by using clinical signs to determine impending death.

Field trials should be conducted on wild rodent infestations and are not considered animal experiments provided the respective tests on efficacy, palatability and humaneness have been confirmed under controlled laboratory studies.

The purpose of Article 62 of the BPR is to minimise the number of tests on animals and not duplicate any studies on vertebrates that might be required by the BPR. While the objective is clear for laboratory tests and semi-field trials, for which animals are used on purpose, for field trials the situation can be seen from a different perspective. Where a field trial is carried out under real life conditions and the rodents subject to such field trial would have been to be killed/controlled in any case by using other authorised products, then it is considered that such field trial does not involve any duplication of testing. Therefore, field trials for PT 14 would be exempted from Article 62 of the BPR.

Concerning laboratory tests and semi-field trials, the objectives of Article 62 (of BPR) would be achieved by data waiving where there were already tests with a fully comparable bait containing an active substance with similar or lower toxicity (see Table 34 in section 5.6.2.2.7 below). In such cases read-across could be accepted provided that, where relevant, a LoA (Letter of Access) is presented by the applicant.

5.6.2.2.2 Laboratory studies for bait products

For testing the efficacy of bait products, two types of laboratory studies are available, mortality tests (i.e. no-choice feeding tests) and choice feeding tests. Since mortality tests give very little information in addition to data from the bait choice feeding testing and in order to reduce the number of animal experiments, mortality tests (i.e. no-choice feeding tests) are not recommended and are not required. However, many applicants may have no-choice studies on their products as they have been conducted in the past. These can still be submitted as part of the data package but no new studies should be conducted.

Tests conducted to EPPO or the specimen protocol (Appendix 13 of this Guidance) are preferable but other data will be considered on their merits. The study must be representative for the treatment. Depending on the intended aim of the product, the house mouse, roof rat, brown rat or other species should be used as the test animal. Wild strain testing is preferable and is most important for the bait-choice test. However, since this is probably impractical for some applicants, an outbreed lab strain (e.g. CD rats) which is likely to exhibit traits of the wild strain is accepted as surrogate.

Rodenticides with special indications, for instance foam products, which are taken up orally but are not bait products since they adhere to the rodent fur, require separate laboratory trials, where the conditions are properly simulated (see section 5.6.2.2.3 below).

The bait choice feeding trials

The aim of the bait choice feeding trials is to determine the palatability of the product for the test animal. If conducted on both fresh and aged product it may provide information on efficacy after a long period of storage of the product (see section 5.6.2.2.5 below). This test is preferably done with wild strain animals. In this test design, animals have the choice between a non-toxic food source (challenge diet) and the bait containing the active substance. Either the amount of bait consumed, in which the active substance is incorporated, or the mortality of the rodents is an indication that the bait is sufficiently palatable for a lethal dose to be ingested. Results are compared with the specified criterion (see section 5.6.2.4.1 below).

Make sure that the challenge diet is a product that the rodent is accustomed to.

Full details of the methods used should be provided and data should be presented to show the daily intake of both untreated diet and product, the palatability ratio (amount of product: amount of challenge diet) or product acceptance (amount of product eaten expressed as a percentage of total (product + challenge diet) consumption) for different sexes of rodent, any signs of poisoning and days to death, with appropriate statistical analysis. When no significant differences exist between the sexes, the data from the two sexes may be combined. Clinical observations should be conducted to determine mode of action, degree of suffering, duration of toxicosis prior to unconsciousness, etc. These data are optional but provide useful information, especially on new active substances.

In some cases comparison with normal food intake is inappropriate. For instance when fast-acting rodenticides cause a reduction in feeding activity or when only very small quantities of bait are required to cause effect. Therefore, the main criterion is not the percentage of consumed bait but the mortality resulting from poison uptake.

Bait choice feeding trials with voles

The test protocol for choice test against voles in the laboratory should be principally the same as for rats and house mice.

5.6.2.2.3 Laboratory studies related to contact rodenticides and gassing agents

Contact rodenticides

The information that should be available in order to demonstrate efficacy will include:

i) Estimates of time to death from individually or group caged rodents exposed to the product for stated periods of time. Reference to EPPO Guidelines (EPPO, 1986) should be made.

ii) Evidence from the laboratory that the target rodents will pick up the required dose from the application method is recommended.

Gassing agents

Rodenticidal gassing agents are typically used in gas-tight buildings, ships, airplanes, containers and storage locations or for burrow fumigation. The type of information that should be available in order to demonstrate efficacy will include estimates of the potency of the active substance and product by inhalation when applied as described in the use instructions in the draft SPC for the product.

There are no internationally recognised standardised test protocols for testing efficacy of rodenticidal gassing agents. In general, the dossier requirements are the same as with bait products. No-choice tests are not necessary. The dossier should include simulated use-tests as

well as field trials. Simulated-use tests should be conducted in gastight containers. The size of the container, duration of exposure as well as the concentration of the fumigant in the container should reflect a real-usage situation.

It has to be noted that the use of gassing agents in sealed rooms, buildings, ships, airplanes or containers (generally denoted here as "rooms") is different from use in burrows (generally denoted here as "rodent burrows"). Hence, it has to be declared for which use an authorisation is applied for. For each type of use a field study must be conducted.

Generally, during each experiment the concentration of gas has to be monitored. The test reports should contain a detailed description of gas concentration, position of measurement points as well as the analytical method. The absence or presence of sorptive materials has to be documented.

Field trials for burrow fumigants should follow the protocol for rodent baits. It has to be demonstrated that rodent populations in infected objects can be eliminated. The study has to include a description of the burrow (location in the infested object, position of entrance holes), for example, Ross, (1986), and Méthode CEB n°254 (2013) listed in Appendix 15 of this Guidance. The methods for a population census before and after application as well as the mortality criteria are the same as for bait products (see Appendix 14 of this Guidance).

Field trials for rooms should include an estimation of the population size, but it is recognised that a feeding census is often not possible (e. g. in containers). In these cases, cages with the respective target organisms (mice, rats) should be introduced to the field object. Their placement should reflect the expected distribution of rodents in the object. It is important that some cages should be placed at spots which would represent "worst case scenarios", i.e. places with air draft (since a room or container may not be perfectly airtight) or in hideouts. The test report should contain a detailed description of placement of the cages, as well as number, age and sex of the test rodents. Exposure time should be according to the use instructions in the draft SPC . After exposure, the number of dead rodents within the sealed room/compartment and/or inside the cages must be determined. Field trials with no scientifically comprehensible data on population reduction or mortality will not be accepted. In cases where a sufficient number of caged rodents have been introduced to field objects for efficacy testing, simulated-use tests can be waived. The mortality criteria are the same as for baits.

Considering the risks linked to the presence of rodents in an airplane, an efficacy of 100% is necessarily required. Indeed rats and mice (these latter being able to hide in places of low volume and completely inaccessible in airplanes) can cause damage, besides the problems of public health, which affect the safety of the airplane and the passengers. Besides possible damage linked to the urine on the electronics, these rodents possess incisors with continuous growth which oblige them to eat away permanently at any type of materials (threads, girdles, steering cables, printed circuits.). There is therefore no tolerance threshold, because a single rodent can cause irreversible damage. In order to make sure that the dose administered according to recommendations and within the framework of fumigation under actual conditions, achieves the required mortality concentrations, the following requirements have to be carried out:

- during fumigation, the measurements of the "CT" (measured effective concentration x time of fumigation) must be systematically taken. The aircraft to be fumigated may not be completely airtight and gas leaks may occur, therefore measures need to be taken for the required 100% efficiency;
- for every trial, the data for the calculation of the "CT" are to be collected from the start of fumigation with statements of concentration (two minimum test points according to the type of airplanes) made at regular intervals (frequency of five minutes) for the duration of fumigation as claimed by the applicant. It is suggested that these data should be collected for two operations of fumigation;
- to make sure that there is good distribution of the gas at lethal concentrations in the entire airplane, rats in individual cages (five rats per test point) must be placed next to all the concentration test points. This will allow estimation of the relation between the measurements, the "CT" and the mortality of the rodents;
- a statement of temperature and humidity should be made.

In case a gassing agent is used in combination with a specific device or is part of a device (e.g., traps), results from laboratory choice tests as well as (semi-) field trials should to be submitted. A no-choice test is not necessary; (semi-) field trials should have the same protocol as field trials for baits. A population census like in bait tests before and after application is needed. The mortality criteria are also the same as for baits.

5.6.2.2.4 Laboratory studies related to specific efficacy claims regarding suitability of bait products for use in damp conditions

Where it is claimed that a product is suitable for use in sewers or under damp conditions, the retention of palatability (such as the effect of the heat and humidity on palatability) should be tested in a choice test[49] against all claimed target species, using product that has been specifically pre-treated to simulate such conditions. Please note that sewers are generally only infested by the brown rat.

For this purpose, the bait product must be exposed to a warm and humid surrounding for at least five days. Bait which is pre-treated in such conditions, may be tested either with experimental animals or, preferably, in a semi-natural test system (pen test). The total number of animals should be 10 to 20.

Below a preferred test protocol is described. Other test protocols will be considered on their merits and are acceptable provided they are scientifically justified.

The bait portions/blocks must be weighed before treatment and then exposed to preferably 30°C to 35°C and 80 to 99% RH for five days. Stable conditions can best be achieved in a climate chamber. The bait should be placed in a water-permeable clay bowl, which itself is placed in a water-tight clay dish. The clay dish contains water, which permeates through the wall of the clay bowl with the bait, so that the surface of the clay bowl is permanently wet to simulate the moist surface of sewer walls. Each pre-treated bait portion/block is applied to the test animals for one day. The bait portions/blocks are then removed and replaced with new pre-treated bait. Since bait exposure to warm and humid conditions is for five days, the baits must be pre-treated stepwise, so that for each testing day, bait with exactly the same pre-treatment time will be applied. The test chamber or test cage is not acclimatised, i.e. the test animals do not experience specifically warm or humid conditions. The bait is replaced daily with freshly pre-treated bait and is offered in a wet clay bowl to maintain surface moisture, so that the bait remains wet and does not dry out during the 24 h exposure to the test animals. Specific acclimatisation of test chambers/cages to high temperatures and humidity is therefore unnecessary and not advisable, as the test animals will most likely originate from laboratory colonies which are kept under normal conditions (i.e. moderate humidity and temperature). High temperatures and humidity may cause them to react with behavioural disturbances.

To determine the bait consumption, bait is removed from the test chambers/cages each day and weighed back. After this, the bait should be dried, preferably by placement in a drying oven at 30 to 36°C (note: since most bait blocks contain a significant portion of paraffin, the temperature for drying must not be too high). Bait portions/blocks are then weighed until no further weight decrease can be measured (i.e. the bait lost all water and is dry).

To calculate the bait uptake, it must be taken into account that the initial weight of the bait is fresh weight, whereas the final weight after bait application to the rats and subsequent drying is the dry weight. Thus, the difference between both is not exactly the amount of bait consumed by the rats, since fresh baits may contain moisture (which adds to the fresh weight at the beginning of the experiment, but is removed after drying for the final weight determination). Hence, the water content of bait must be determined by placing five untreated bait portions for each product in a drying oven until no further weight decrease is determined. The difference between the fresh and dry weight is then taken into account for the determination of the amount of bait uptake (Equation [1]):

$$[1] \quad b = f - \frac{d}{(1-w)}$$

Where:

[49] Field tests may be accepted in case of a controlled situation without re-entry of rats, but laboratory studies are preferred.

b is the amount of bait taken up

f is the fresh weight of the bait prior to heat and humidity exposure

d is the dry weight after bait application, consumption and drying

w is the proportion of water content of the bait (determined through drying of untreated bait).

The relative portion of bait taken up by the test animals in relation to overall food consumption can be then calculated as (Equation [2]):

$$[2] \quad c = \frac{\Sigma b}{\Sigma b + \Sigma a} \times 100$$

Where:

c is the percentage of consumed bait during the test

b the amount of bait taken up (corrected after Equation [1])

a is the amount of challenge diet taken up.

5.6.2.2.5 Studies related to specific efficacy claims regarding to the shelf life of bait products

When a bait product is claimed to be effective after a long period of storage, it is necessary to demonstrate that the product will still be effective and palatable after the stated storage period (i.e. shelf life). Analytical studies on active substance content are therefore not sufficient to support shelf life claims of bait products.

Based on expert opinion, most bait products have been found to be effective and palatable for 24 months (with preservatives) . Efficacy testing should therefore only be provided for:

- bait products with preservatives that claim a shelf life of longer than 24 months;
- bait products without preservatives that claim a shelf life of longer than 12 months;
- bait products for which the degradation of the active content is >10% and assessment of the degradation on the efficacy is needed to substantiate the shelf life claim

For bait products with a shorter shelf life claim than stated above, no efficacy tests on aged bait (i.e. product at the end of maximum storage) have to be provided. For these products it is sufficient to provide tests on fresh bait (i.e. newly produced product).

For bait products with a longer shelf life claim, the applicant must deliver data on the palatability of the product at the end of maximum storage for all target organisms claimed. The palatability of the aged product preferably is tested in bait choice feeding trials, but can be tested in field trials, provided these tests are scientifically valid (see section 2.6 below). Accelerated ageing studies, i.e. palatability studies in which the product tested is stored under challenging conditions, are not acceptable as these cannot simulate longer storage periods.

5.6.2.2.6 Field trial and semi field trial

The following text describes the field and semi-field testing of bait products, but is also largely valid for other rodenticide products.

Field trials

The aim of the field trial is to demonstrate the results on the effectiveness (palatability, mortality and humaneness) obtained during laboratory studies of the rodenticide product containing active substance under actual use conditions for the purposes of marketing authorisation. Field trials should only be performed once efficacy, palatability and humaneness have been confirmed in laboratory (semi-field) studies under Directive 2010/63/EU.

Tests conducted to EPPO or the specimen protocols (Appendices 13 and 14) are preferable but other data will be considered on their merits. Depending on the intended use(s) of the product, populations of the respective target organisms (house mice, brown rats, roof rats or others) are used for this trial.

Ideally, sites chosen for field trials should be representative of the range of locations where the rodenticide is to be used (indoor/outdoor), and should be infested with sufficient numbers of the target rodents so that the effectiveness of the product can be clearly demonstrated. It is

advantageous if the rodent infestations on the sites chosen are, as far as possible, discrete and not subject to potential rapid re-invasion. Rodent activity on the site should be determined before and after treatments using at least two standard techniques.

Sketch maps of the sites approximately to an indicated scale showing all the important features including signs of infestation and location of rodenticide application should be provided. The amount of bait applied at each bait point and the distance range between bait points should correspond to those given in the draft SPC. Replenishment of the bait should follow intervals given in the draft SPC . Bait exposure should normally be for 4 days for acute products and 30-40 days for multi-dose products after the first bait uptake or less when full control is achieved. Data should be presented to indicate levels of rodent activity both before and after treatment, amounts of bait consumed and all relevant information regarding treatment details.

Semi-field trials

As an alternative or addition to 'field' trials, evidence of the efficacy of a rodenticide product may be obtained with semi-field trials (otherwise referred to as pen trials). A semi-field trial simulates field conditions under controlled laboratory conditions. Bait acceptance and bait uptake in the field is strongly influenced by the social behaviour of the target species. Both rat species (*R. norvegicus* and *R. rattus*) as well as house mice (*M. musculus*) are social animals, and food exploration is largely social in these species. Hence, the most important field condition to be simulated is the presence of conspecifics, i.e. the semi-field trial has to be conducted with groups of rodents. Group size should be at least 10 animals in tests with both rat species and at least 10 animals in tests with house mice. Sex ratio should be approximately 1:1 although single sex groups may be used with robust justification, e.g. to avoid unacceptable levels of aggression. Groups should consist of related animals to avoid intraspecific aggression. The test animals should either be directly caught in the field, or be bred from wild catches, as only wild-strain rodents show the typical behaviour of the target species which could be expected in the field. A test with laboratory strain rodents cannot be regarded as a proper simulation of field conditions.

The test arena should provide shelter for the animals, as well as sufficient space for the animals to roam. The minimum space requirement would be ≥ 0.5 m² per rat and 0.25 m² per mouse. If possible, cage enrichment such as branches, ladders, tunnels and wooden nest boxes with nest material may be provided and details on this should be given in the test report. Cage enrichment should be designed in a way that daily inspection for dead rodents and spilled bait material and feed causes only minimum disturbance.

The rodents have to be familiarised for at least three days with the test arena prior to bait exposure. The semi-field trial is always a choice test, and a suitable challenge diet must be provided together with the bait. The amount of bait applied should correspond to the amount given in the draft SPC. Bait exposure should normally be for 4 days for acute products and 30-40 days for multi-dose products. Bait exposure must be followed by a 14 day post baiting observation period.

Field trials with voles

For efficacy testing of products against voles, the test protocols for house mice and rats are only suitable when the infestation is inside a building. Efficacy testing outside of buildings should be conducted with a specific protocol. In contrast to rats and house mice, voles excavate and inhabit galleries (tunnels beneath the surface) for food exploration and nesting.

For each field trial with voles, one test plot and one control plot should be investigated. Principally, the test protocol is the same for oral baits and gassing tablets/pellets. The pre-treatment and post-treatment censuses are conducted by counting occupied galleries. For this, at least ten galleries should be opened on each plot (treatment and control). After 24 h, the number of refilled galleries is then counted. The number of refilled single openings is set into relation to the number of openings as an indicator for vole activity. Depending on the vole species, an alternative census method could be the closing of burrow openings. Reopening of burrows is then counted as a sign for activity. During the treatment, vole activity should be controlled after 5 and 10 days with the same method.

Application of the rodenticidal product should follow the use instructions in the draft SPC. Normally, one bait portion has to be placed in each gallery. Replenishment of the bait should

follow intervals given in the use instructions in the draft SPC. Bait exposure should be for 14 days. The efficacy is then calculated as (Equation [3]):

$$E = 100 * \left(1 - \frac{t2 * c1}{t1 * c2}\right)$$

Where:

E is the efficacy,

t are treated plots

c are control plots,

t1 and *c1* are the ratios of refilled galleries/open galleries before treatment

t2 and *c2* are the ratios of refilled galleries/open galleries after treatment.

Treatment and trials with oral bait should be undertaken in spring or autumn, as in the winter not much activity is to be expected, and in summer other food sources than the bait are too abundant.

5.6.2.2.7 Waivers

Waiving of laboratory trials or semi-field trials will reduce animal testing. For bait products, because the composition of the bait determines the palatability and hence efficacy of the product, even small changes in ingredients may affect the attractiveness. This may differ between target organisms and is difficult to predict in advance.

Semi-field trials

Laboratory testing of bait products (bait choice test or semi-field trial) should always be requested for new active substances, or if a product was altered regarding the active substance concentration and/or bait formulation. One exception would be if there were already test data with a fully comparable bait, i.e. containing a different active substance but otherwise the same or similar formulation with the same mode of action and similar or lower toxicity; (see Table 34 below for a ranking of toxicity of existing active substances), in such cases read-across could be accepted; however if the two formulations contained the same active substance, then the concentration of the active substance would need to be the same.

Field trials

Field trials are always required when the composition of a product is changed. Exceptions could possibly include changes of minor importance in ingredients that are likely not to have an effect on palatability or efficacy, such as change in colour of a product. In case of waiving, the applicant needs to provide a robust justification why no testing was performed.

Read-across between species is generally unacceptable unless the applicant can demonstrate that there is no significant difference in the susceptibility and behaviour of the species.

Table 34: Toxicity ranking of known active substances used in anticoagulant rodenticides based on LD 50 (acute) data of brown rats and house mice compiled from CA-Reports, ranking from high (1) to lower toxicity (3)

Rank of toxicity	Active substance
1	Flocoumafen, brodifacoum, difethialone
2	Bromadiolone, difenacoum
3	Chlorophacinone, warfarin, coumatetralyl

5.6.2.2.8 Biocidal Product Families (BPF)

A BPF of rodenticide baits may contain several bait products with different formulations, for example, various grain, block, paste and gel products. Each bait formulation should be

allocated to a different meta-SPC[50]. Each bait formulation within the BPF has to be tested, because it cannot be predicted which form is the least palatable. It would also be difficult to select one product that could be regarded as a 'worst case scenario' for testing all the formulations. Within a given meta-SPC, an individual product should only be tested to consider the minimum level of efficacy within the concentration ranges of the active substance in that meta-SPC.

5.6.2.3 Methodology of assessment

There are many standard test methods currently available that may be appropriate for the assessment of the effectiveness of rodenticides. A list of such test standards is presented in Appendix 15 of this Guidance.

In addition to the standard test methods presented in Appendix 15, specimen protocols for a choice test and a field trial are presented in Appendices 12 and 13 respectively. These Appendices are intended only to provide further information regarding the types of studies that may be utilised to assess the efficacy of some rodenticides, and some of the factors that should be taken into account.

Any known limitations on efficacy (including resistance) should be considered during the assessment. Possible restrictions, risk mitigation measures, or recommendations concerning the use of the product in specific environmental or other conditions can be considered. Possible factors that can reduce the efficacy, for instance hot, cold or humid environments or the presence of other substances, in addition to the grounds for these should be stated. Possible recommendations concerning the avoidance of the continuous use of the product in order to prevent the selection and spread of resistant strains and the grounds for these (see TNsG on Product Evaluation and a report on risk mitigation measures for anticoagulant rodenticides as biocidal products [51)] . State if the product cannot be mixed with, for example, other biocidal products or if the use of the product with other biocidal products is recommended. The guidance given on resistance for the corresponding data requirement of the active substance also applies here. The study results are compared directly with the criteria for efficacy (see section 4.1 below).

5.6.2.4 Assessment of authorisation

5.6.2.4.1 Norms and criteria

In accordance with Article 19(1)(b)(1) of the BPR, a biocidal product may only be authorised if it is sufficiently effective. This is implemented in the following way.

In general rodenticide products are normally considered to be sufficiently effective if the following results can be achieved:

- required results in laboratory test and semi-field trial:
  - ≥90% mortality within a relevant time frame
- required results in field trial:
  - Monitoring of the test population should show a ≥90% decrease of the population

Rodenticide bait products are considered to be sufficiently effective if the following results can be achieved:

- required results in the bait choice feeding test, semi-field trial and sewer test (if claimed):
  - ≥90% mortality. The percentage of ingested bait containing the product should be normally ≥20%, but it may be lower because a mortality of ≥90% the product would still be effective. In case of a bait ingestion <20%, justification should be provided.
- required results in field trial:
  - feeding on census bait after treatment should be reduced by at least 90% from the

[50] See Q&A pair number 6 in Annex IV of the Note for guidance "Implementing the new concept of biocidal product families" (CA-Nov14-Doc.5.8 – Final.rev2). [https://circabc.europa.eu/w/browse/c309ae58-bdd7-421d-a678-8d8ac361d4e0]

[51] "Risk mitigation measures for anticoagulant rodenticides as biocidal products" [https://circabc.europa.eu/sd/a/343a61cd-b8d4-40af-9e5c-4f763aea3240/CA-Nov14-Doc.5.1%20-%20draft_final_report_RMM.docx6].

levels of feeding on census baits before treatment. When other types of quantitative monitoring of the test population are used, such as tracking activity measurement and census by trapping, they should sufficiently show the decrease of the population (≥90%).

The efficacy of the product after a specified storage time (e.g. shelf life as claimed in the use instructions in the draft SPC) is also taken into account when assessing efficacy of a rodenticide bait.

Deviations from the norms are possible, but must be justified in the application. The Competent Authority will evaluate any justification on a case-by-case basis, consulting the other CAs where appropriate and decide whether it is acceptable or not.

In order to promote the development of new types of products (less toxic, more humane), a mortality <90% could be acceptable when the product is used as an accompanying method, (i.e. used with another product to demonstrate efficacy), but not as a stand-alone product. However, mortality of these new type of products should not be <50%. The use of a product as an accompanying method should be reflected in the use instructions in the draft SPC.

For the assessment of resistance, reference is made to TNsG on Product Evaluation . Information on resistance testing techniques is also available from the Rodenticide Resistance Action Committee (RRAC)] and Prescott *et al*. (2007).

5.6.2.5 References for PT14

Prescott, C. V., Buckle, A. P., Hussain, I. and Endepols, S. (2007) A standardised BCR resistance test for all anticoagulant rodenticides. International Journal of Pest Management, 53 (4). pp. 265-272. ISSN 0967-0874.

Rodenticide Resistance Action Committee, RRAC. A Reappraisal of Blood Clotting Response Tests for Anticoagulant Resistance and a proposal for a standardised BCR Test Methodology [www.rrac.info].

Ross, 1986. Comparison of fumigant gases used for rabbit control in Great Britain. Proceedings of the Twelfth Vertebrate Pest Conference (1986). [http://digitalcommons.unl.edu/cgi/viewcontent.cgi? article=1053&context=vpc12].

5.6.3 PT15 Avicides, PT16 Molluscicides, vermicides and products to control other invertebrates & PT17 Piscicides

Please refer to the General sections 1-3 of this guidance and the TNsG.

For product-type 16, EPPO guidelines for efficacy testing are highly recommended (e.g. EPPO guidelines 95 for molluscicides in terrestrial environment).

5.6.4 PT18 Insecticides, acaricides and products to control other arthropods

5.6.4.1 Introduction

Depending on its field of use a product to control insects and other arthropods may be classified as a biocidal product or plant protection product. This section covers the products to control insects and other arthropods in the category of biocides, which are products against all pest arthropods except those that are plant parasitic.

This first section gives a general introduction. The following sections describe per insect or per type of use what the requirements for efficacy testing are. Information is missing on some of the organisms to be controlled with these products and also some of the uses and types of products. For instance, little information is provided on treated articles (e.g. insecticide treated mosquito nets etc.). These data gaps will be filled in a future update of this guidance.

5.6.4.1.1 Aim

The aim is to assess the efficacy of biocidal products, to ensure that only effective products enter the market.

5.6.4.1.2 Global structure of the assessment

A full assessment of efficacy is conducted for applications for product authorisations.

Factors, which are taken into consideration during assessment of the efficacy for a biocidal product to control insects and other arthropods for which authorisation is sought, are:

- the target organism to be controlled;
- the physical state in which the product is applied (e.g. liquid/powder/bait);
- the areas of use, these may be:
- in and around residential homes and other spaces in which people are accommodated;
- in and around spaces in which animals are accommodated
- in spaces intended for the preparation, processing or storage of food and beverages;
- in empty stores, ship's holds, factories and silos.

Information on effectiveness and intended uses of the product, together with its active substances, must be sufficient to permit an evaluation of the product, including the nature and benefits that accrue following use of the product in comparison to suitable reference products or damage thresholds, and to define its conditions of use.

A combination of laboratory studies, rigorous simulated-use laboratory studies, or field studies can be used to evaluate whether the product is effective for the requested use(s) at the specified doses. Data from these studies are compared with the specified criteria.

Assessment will be made mainly in relation to the claims for the effectiveness of the product made on the product label. This assessment will take into account the pest(s) to be controlled, indoor or outdoor use, the method(s) of application, application rates and use patterns of the product, maximum storage period of the product, together with any other specific claims made for the product. More information on different aspects of the label claim can be found in Appendix 1. Appendix 17 shows examples of possible label claims.

5.6.4.1.3 Dossier requirements

Data on efficacy are required for every application for authorisation.

The following guidance is designed to be flexible and does not specify rigid protocols to which tests must be conducted. Published or unpublished data from any source will be considered provided the data are valid and relevant to the application. In all cases, the methods and results have to be described in sufficient detail to make the data reproducible and to allow a full assessment. Anecdotal evidence will not be acceptable.

Ideally, data should be generated using internationally recognised testing methods (ISO, CEN, OECD, WHO etc.). Several international standard test methods currently exist for insecticide/acaricide products. A list of these is presented in Appendix 18 to this document.

If there are no guidelines available or guidelines are not suitable, the applicant may use their own methods (intra-company Standard Operating Procedures), on condition however, that the studies are scientifically robust, well reported and provide a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. The use of existing guidelines, with revisions to make the guideline more suitable for the specific product or company conditions, is also possible.

For each test information such as the following should be available:

- the names of actives substances and their respective concentration in the tested formulation;
- as the formulation may be very important for the efficacy, if the test item differs from the product to be authorised, its composition should be provided;
- a statement about what is expected from the test, what should be determined and with which precision. Power and sample size considerations should be included as well;
- description of the test conditions (size of cage, floor area, presence of harbourages, presence of (alternative) food, water, temperature, photoperiod, location, weather conditions);

- are the test organisms allowed to acclimatise to the test conditions before the test? For how long?
- how many test organisms are present (sample size)?
- describe population composition (males, gravid or non-gravid females, nymphs, larvae, age of the population or generation number F1, fed or unfed) noting that the feeding behaviour of some insects (i.e. *Blattella*) changes during their life;
- are the test organisms starved prior to the test?
- are field strains or known insecticide-resistant strains tested (claim "effective against strains resistant to x")?
- a description of the history and origin of the test strain;
- is bait consumption determined? If so, a covered bait should be included to determine weight loss due to evaporation to correct weight loss of the exposed bait for actual consumption;
- are one or more alternative baits (e.g. registered reference products) or alternative food source present in the same test container or protocol?
- raw data should be available for each study, rather than just a summary of the results;
- show the results of both tests (with biocide) and control (without biocide) treatment, preferably in a table;
- size of the test population in the field before and after the test;
- description of the monitoring methods used before, during and after the test;
- statistical methods, if appropriate.

5.6.4.1.3.1 Test design

Although in general nationally or internationally recognised testing methods are preferred it is not always possible to use these. For some products no standard methods are suitable. In that case a test has to be designed.

Various factors must be considered when designing the tests, for example the number of test individuals (insects, mites, other arthropods) needed. The ultimate aim of relevant considerations should be to design experiments that economise on test individuals, but on the other hand generate sufficient power to detect effects of a magnitude considered important to demonstrate. To save test individuals, replicate tests are conducted. Another argument for using replicates is to account for the variation among test individuals in susceptibility and responses to the biocides. Numbers of test individuals per replicate group and dose level (treatment group) as well as the number of replicates in the entire study need to be established prior to conducting the tests. As the improvement in power wears off substantially as the number of replicates increases beyond five, it is usually sufficient to conduct four or five replicate tests at each dose level, employing 10 (or 20) test individuals each. The precise needs will depend on the size of the variances, relative and absolute, between and within the replicates. This can differ between insect species and test design. Sample size should be adequate to detect differences among groups (untreated vs treated) with a statistical power of at least 80%. Some details on these issues are outlined at the end of each section.

Useful information on the principles of test design, analyses end evaluation of efficacy trials can be found in the EPPO standards pp1/152(3) and pp1/181(3).

5.6.4.1.3.2 Test examples

In the following sections (5.6.4.1.3.2 to .15) examples are given of what kind of tests can be expected for efficacy testing. Sometimes these examples are a summary of a standard test, in other cases a company test is described or a general idea of what the test should be like is given. There is a great variation in how specific the description is. For instance, the number of replicates is given only when this was determined in the test described.

In all cases these tests are only meant as examples, not obligatory requirements. Since products against insects and other arthropods are so diverse in application method, mode of action etc. the guidance cannot possibly cover all possible ways of controlling arthropods.

5.6.4.1.3.3 Laboratory versus (semi) field trials

Laboratory and field trials with the test arthropods are normally needed to assess the efficacy of the product. Field trials are not mandatory in some cases, as outlined in the sections on specific groups of arthropods below. In some cases when robust field studies are available, laboratory studies can be waived. If the product is applied as a bait, the entire bait, including the bait-box if applicable, should be tested, not only the product which is contained in the bait. When efficacy against several insects or other arthropods is claimed not all organisms have to be tested when appropriate bridging studies are available.

In the case of field trials where true replication is almost certainly impossible to achieve, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

In the following sections (5.6.4.1.3.2 to .12) more specific dossier requirements are given per pest species. In most cases a general description of a proposed method is provided. This is only to give an idea of what kind of tests should be provided. More detailed descriptions of tests can be found in the standard test methods (norms) listed in Appendix 18. This is a list of all available methods (as far as we know now) without distinction on usefulness, repeatability, order of acceptability or robustness. Some norms might have a different approach than described in the section for that insect. If this approach is more suitable for the product under investigation the norm should be used.

5.6.4.1.3.4 The importance of controls on efficacy studies

The importance of control experiments for efficacy studies must be stressed with regard to the efficacy evaluation. Studies should be conducted alongside negative controls wherever possible to provide a reference point for the treatment results. A useful definition of this term is given: "A negative control situation may be one in which the experimental design of the study is identical to that of the biocide challenge test except that the biocidal agent is not applied in the control study. A biocidal agent may be considered as the formulation or as the actual biocidal active ingredient itself."

The negative control trial should normally be of similar size (i.e. number of replications) as the test itself, to make statistical comparison possible and to get a fair impression of control mortality.

A relevant reference product (authorised, commercially available) can often be included at label rates in a protocol for laboratory and/or field studies as positive control. Unfortunately at this moment no standard reference products are available, however, an authorised reference can be included.

It is recognised that generation of such control data can be relatively straightforward in well-defined test situations such as laboratory and simulated-use tests. However, it is also recognised that this can present a problem in field situations, where control sites may not be environmentally equivalent to the treatment site.

In such instances, there may be an alternative means of generating reference data other than collecting data from an untreated site. This method may involve pre-treatment monitoring of the site in question. This monitoring must be quantitative, e.g., assessment of numbers of trapped insects. In these instances, a 'baseline' infestation level would be established through such monitoring and then the effect of treatment on this baseline can be assessed. Post-treatment monitoring is required for this method.

5.6.4.1.3.5 Specific data to support label claims

In assessing the efficacy of a biocidal product to control insects and other arthropods competent authorities should in particular take the following parameters into account:

- target organisms/spectrum of activity;
- mode of action/effect;
- use patterns/methods of application;
- dose rate.

The data provided in support of the efficacy claims must be sufficient to cover these key parameters.

5.6.4.1.3.6 Examples of specific label claims with respect to target organisms

For specific target pests where only efficacy against one insect/arachnid order or a certain family within that order is claimed, data against only a limited number of pest species will normally be required. To illustrate this point, a number of examples are given below:

- FOR USE AGAINST FLEAS - Data against the cat flea (*Ctenocephalides felis*) or the dog flea (*C. canis*) should normally be available;
- FOR USE AGAINST COCKROACHES - Data against two key species such as German cockroach (*Blattella germanica*) and the oriental cockroach (*Blatta orientalis*) should normally be available;
- FOR USE AGAINST DUST MITES - Data against *Dermatophagoides sp*. should normally be available.

In the European tropical overseas regions, the most common genus encountered could be different. A specific claim should therefore be proposed, with referred target organisms. This special request could concern for examples termites, cockroaches or mosquitoes.

5.6.4.1.3.7 Examples of broad label claims with respect to target organisms

Broad label claims, such as "crawling insect killer" or "flying insect killer", should be accompanied by qualification of the range of pests against which the product may be used. When broad claims are made, data on representative pest species will need to be provided for the range of pest orders against which efficacy is claimed.

Representative pests from these orders will have to be appropriate to the use pattern of the biocidal product i.e. the environment of the areas to which the biocide is to be applied and the nature of the application (e.g. whether it is a space application or surface application) will define the most appropriate pests to be tested.

For each order stated, at least the principal target species will need to be tested for public hygiene use, before a general claim is likely to be supported. In more specific areas, such as use against stored product pests, data on at least two major representatives of the orders in question will normally be needed before a general claim is likely to be supported.

Where such a claim covers a diverse range of pest habitats and pest morphology and biology, data from a greater number of representative species will need to be provided. Appendix 17 shows examples of possible label claims and the test species required.

When cockroaches are used as a reference species, it can only be used for the general claim “crawling insects”. If efficacy against other insects are claimed specifically (e.g. crawling insects including bed bugs) tests against these other insect should also be provided. Also if a company wants authorisation for more specific use with the same product they have to present specific data on the specific pest they are claiming. This is a consequence of the use of “reference species”, which should not be a way of short-circuiting the evaluation for efficacy.

5.6.4.1.3.8 The distinction between professional and consumer products

In some cases the dossier requirements and norms and criteria for the evaluation may differ between professional and consumer products. Products used by professionals must have a high level of efficacy since the objective is to eradicate the infestation. For consumer products an immediate knockdown is often more important than eradication, of course depending on the claim. For instance a spray against cockroaches does not necessarily have to eradicate the whole population but it should work fast. Consumers want to see that the insect/arthropod dies/knocks down immediately after they spray. For consumers it is difficult to eradicate a whole cockroach population since reinvasion from other premises will take place, therefore eradication does not always have to be proven. For each pest group it will be listed whether requirements differ for consumer and professional products.

5.6.4.1.3.9 The distinction between principal target and secondary/incidental target pests

Screening tests (see sections below for details) can be used as bridging studies, showing similar effect of the product to different pest species, after which in some cases field studies can be waived for secondary target pest species.

5.6.4.1.3.10 Claims for residual efficacy

Most insect/arthropod pests are cryptic and/or nocturnal in behaviour and are unlikely to be contacted directly by a spray during application. For this reason many control programmes involve the use of relatively stable active substances applied to buildings and other surfaces to leave residual deposits. These compounds are intended to remain chemically active and therefore effective for periods of weeks up to several months following treatment, i.e. they have a high residuality. Residual life is a term to describe the period during which the biocide will be present in sufficient quantity to kill target pests, which walk upon it for a sufficient period of time to pick up a lethal dose.

Thus the amount of biocide residue deposited on treated surfaces is critical to the effectiveness of many treatments against crawling (and flying) pests. Ideally, the amount of residue deposited should be determined for instance by calculation or under actual or simulated-use conditions. The method(s) of determination must be available with the test data.

Residual efficacy must be proven in tests. Usually, laboratory testing is performed to establish the efficacy direct after application and at the end of the residual life of the product.

The types of surfaces to which residual products are applied must be reported since surface type has a pronounced effect on the amount of active residue available to pests. In general a selection of both absorptive and non-absorptive surfaces, related to the label claim, should be tested when supporting a residuality claim for crawling (and flying) pests. These could include vinyl tile or linoleum, stainless steel, painted and unpainted wood, carpet, concrete and ceramic tile.

Efficacy data submitted to the competent authority in support for residual treatments should indicate the appropriate dosage and the utility of the formulation when used as directed.

5.6.4.1.3.11 Residual treatments may also involve the use of palatable baits.

When a bait product is claimed to be effective after a long period of storage, it is necessary to demonstrate that the product will still be effective and attractive after the stated storage period. The applicant must either submit data for palatability of the product at the end of maximum storage or alternatively (in case of a new product) data for a stress test with 'accelerated ageing', i.e. a palatability test with the product which is stored under challenging conditions (see FAO accelerated test).

5.6.4.1.3.12 Claims relating to outdoor use

When products are intended for outdoor use, tests should normally demonstrate efficacy under outdoor conditions. Changes in temperature and rainfall can have effect on the efficacy of the products. In general field trials cover this outdoor use. In some cases a field trial can be waived when a laboratory test can be done under worst case conditions.

5.6.4.1.3.13 Mode of action

There are a variety of modes of action and possible effects on target organisms derived from the proposed use of a product to control insects and other arthropods. The available data should give brief details to indicate the route and nature of the action (e.g. whether action is by contact or stomach poison), and the nature of the effect (e.g. cholinesterase inhibitor, chitin synthesis inhibition, juvenile hormone analogue giving rise to sexually immature adults or supernumerary nymphs).

A variety of molecules exist which control invertebrate pests by preventing successful completion of the insect's life cycle, rather than being acutely toxic to the insect. Examples of such molecules include chitin synthesis inhibitors (CSI) and juvenile hormone analogues (JHa). The CSI act by disrupting the deposition of chitin during the formation of the insect’s larval cuticle after moult, whereas JHa aim to interfere with the hormone based control of metamorphosis and reproduction. These two types of molecules are often referred to as insect growth regulators (IGR) to distinguish them from conventional insecticides with neurotoxic action.

Consequently molecules that affect the developmental cycle of insects may be effective without resulting in the immediate death of the insect and therefore efficacy trials should be designed to address the most appropriate life cycle stage of the insect sensitive to the molecule of interest and also to measure any long term effects (e.g. on the fertility and fecundity of females or any effects on the embryonic development in the egg stage).

For example, in measuring the effectiveness of JHa, trials should be designed to record the number of adults produced from treated nymphs/larvae, the number of adults with deformed wings or terminalia and the mortality of insects prior to and at metamorphosis. Additionally a number of newly moulted females should be selected randomly from each treatment dose/formulation and their ability to produce viable eggs/oothecae after pairing with untreated males should be recorded.

IRAC, the Insecticide Resistance Action Committee, has developed a classification of insecticides based on mode of action (www.irac-online.org).

5.6.4.1.3.14 Resistance

Information on resistance and the likelihood of its development is required for BPR Annex I inclusion and is also important for product authorisation.

For insecticides resistance can be a problem. Some pests are more capable of building up resistance then others. For instance flies, with multiple generations and multiple females that can lay many eggs, resistance can be expected to build up easily. In ants on the other hand, with one or few queens who lay eggs for a long period, and a biocide that kills the whole colony most of the time, it is not to be expected that resistance will build up. Therefore, a resistance management strategy has to be provided for flies but not for ants for evaluation at product authorisation.

A resistance management strategy is generally based on the use of two modifiers, the frequency of use and the rotation with other active substances. For instance, for products against house flies, a label could state that the product should not be used more than five times per year and should only be used in rotation with at least one other product with a different mode of action.

For consumer products it is necessary to make clear that there might be a risk of building up resistance and that this can be reduced. Since consumers have no knowledge of resistance the label claim should contain information to prevent it. For instance, the following sentence could be added to the label: "When the product is not used according to the label resistance of insects might occur. When the infestation persists contact a professional."

More information on resistance can be found in Chapter 6.2 of this TNG on Product Evaluation and the Insecticide Resistance Action Committee (IRAC: www.irac-online.org).

5.6.4.1.4 Methodology of assessment

Methods of application and dose rates

When considering the overall evaluation of a proposed label claim competent authorities should ensure that the data presented are relevant not only to biological challenge and treatment environment but also that the method of application and application/dose rate(s) used in the test(s) are appropriate to the label claims and proposed use of the product.

The application technique should therefore reflect the claims proposed on the label, whether crack and crevice, spot, space spray, contact spray or total release.

General considerations

The efficacy data submitted should demonstrate that the biocidal product, when used as directed by the product label, will result in a measurable beneficial effect. The data supplied should demonstrate that an acceptable, consistent level and duration of control or other intended effect will result from the use of the product at the recommended dose rate.

This may, depending on the individual product, be measured as a reduction of the pest population to an acceptable level or a reduction in damage. The acceptable level may vary depending on the purpose of the proposed use.

Competent authorities should evaluate available data to determine whether they are sufficient to support a label claim.

The competent authority will examine the submitted data package and a judgment will be made as to whether any data omissions are considered significant as to delay assessment. Those so identified will be communicated back to the applicant. The applicant can then supply additional data or modify their label claims in line with whatever has been supported.

Any known limitations on efficacy (including resistance) should be considered during the assessment.

- possible restrictions or recommendations concerning the use of the product in specific environmental or other conditions. State possible factors that can reduce the efficacy, for instance hot, cold or humid environments or the presence of other substances, in addition to the grounds for these. Possible recommendations concerning the avoidance of the continuous use of the product in order to prevent the development of resistant strains and the grounds for these (see also TNsG on product authorisation Chapter 6.2). State if the product cannot be mixed with, for example, other biocidal products or if the use of the product with other biocidal products is recommended;
- the guidance given on resistance for the corresponding data requirement of the active substance also applies here.

5.6.4.1.4.1 Assessment of specific claims

Sometimes a claim will include specific properties of the product, for instance:

- kills within 15 minutes;
- residual effect up to 3 months;
- storage period up to 5 years;
- control of tropical ants.

Where a particular property is claimed the data submitted to support the product should show that the product actually has these properties. If data do not support this claim, the product may still gain authorisation with amended label claims, provided that the product still shows acceptable efficacy.

For example: If a product claims complete control of ants within 2 weeks of application, the data submitted must show a high level of mortality (approximately 100%) within two weeks of application in order for these claims to be acceptable.

However, if the submitted data showed 90% mortality within 2 weeks and 100% mortality within 3 weeks, the product may still gain authorisation provided that the product claims were amended to 'complete control of ants within 3 weeks of application'.

Situations such as the example above will require each study to be evaluated on its own merits, taking into account what the data is actually showing. Evaluators must use scientific judgement to determine when authorisation would not be acceptable.

For example:

> If a product claims to kill ants within 15 minutes of application, the data submitted must show sufficient mortality within 15 minutes of application in order for these claims to be acceptable.

However, if the submitted data showed 50% mortality within 15 minutes but 90% mortality within 2 hours, the product would still not be granted authorisation on the basis that for claims such as 'kills ants', the average user would expect a rapid visual effect following application (unless the product label clearly states how long the product takes to have an effect).

5.6.4.1.5 Assessment of authorisation

When considering the overall evaluation of proposed label claims, competent authorities should ensure that the data and the method of application and application/dose rates used in the tests are appropriate to the label claims and proposed use of the product.

5.6.4.1.5.1 Norms and criteria

The test results are compared directly with the norms and criteria for efficacy described below per insect/arthropod pest. The performance criteria set in this guidance ask for high levels of efficacy, which is of course what we aim for. However, some products that do not fully meet the criteria can still be valuable in some cases.

When a product does not perform to the criteria it should be justified in the application why this product is still recommended for authorisation. For example, in a field trial the criteria may not be met because of immigration of insects from untreated areas (e.g. flies, mosquitoes). When this is explained well in a justification the product might still be accepted for authorisation, depending on the results of other field trials, simulated-use and laboratory tests.

Special attention should be paid to resistance, since under low insecticide pressure resistance can build up more easily. Moreover, it should be taken care of that no placebo's or misleading products are registered. If the efficacy level is significantly lower than the criteria state it should be mentioned on the label.

The justification will be evaluated case by case. The product should not be authorised, unless there is a good reason for having a product of lower effectiveness.

5.6.4.1.5.2 Assessment

The assessor/expert assesses on the basis of the label claim and the above criteria. If the product was assessed to be sufficiently effective in laboratory tests and/or field trial, it will be authorised as far as efficacy is concerned.

5.6.4.2 General Claims: Crawling Insects, Flying Insects, Acaricide

5.6.4.2.1 Introduction

Some products have a very broad claim: against crawling insects, against flying insects, insecticide-acaricide spray, etc. In these cases it is not possible to test the product against all claimed target pests. For each group claimed tests should be performed on a few relevant species, of significant importance, and on the species specifically claimed on the label.

General claims (e.g. insecticide, crawling insects) cannot be used for bait products, since the bait differs per insect species.

5.6.4.2.1.1 Crawling insects

A crawling insect is defined as an insect that generally moves on the ground. These include amongst others cockroaches, ants, fleas, crickets, silver fish, bed bugs and carpet beetle larvae. The effect of biocides on these insects is primarily based upon contact. The products involved can be sprays, dusts, etc. Amongst the crawling insects, cockroaches are the most difficult to control.

5.6.4.2.1.2 Flying insects

A flying insect is defined as an insect that generally flies from one spot to the other. These include flies, mosquitoes, wasps and moths. The products involved can be sprays, strips, paints, etc.

5.6.4.2.1.3 Insecticide, acaricide and other arthropods

A general claim for insecticides includes all insects. A general claim for acaricides includes ticks and mites. Other arthropods could include spiders (Araneae), harvestmen (Opiliones), centipedes (Chilopoda), millipedes (Diplopoda), woodlice (Isopoda) and scorpions (Scorpiones).

5.6.4.2.2 Dossier requirements

A clear label claim should be submitted. The study results of trials should demonstrate the efficacy of the product based on the submitted label claim. Laboratory, simulated-use tests and field trials with the test organisms are needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. Ideally, data should be generated using national or international recognised testing methods (ISO, CEN, OECD, etc.) where available and appropriate. See Appendix 18 for a list of available guidelines. If there are no guidelines available or guidelines are not suitable, the applicant may use their own methods (intra-company Standard Operating Procedures), on condition however, that the study is scientifically robust, well reported, provides a clear answer to the question and demonstrates the efficacy claimed. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. A control treatment without biocide (negative control) should be included in all laboratory trials.

In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single biocidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history, season, etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.2.2.1 Test species

Claim: crawling insects. In case of an application for authorisation of a product with a claim of "killing crawling insects" a product, which has demonstrated sufficient effectiveness against cockroaches, may also be authorised to control other crawling insects. However, if also population control and/or nest kill is claimed both cockroaches and ants have to be tested.

Tests with cockroaches should normally be performed with two key species, one small, one large, such as the German cockroach (*Blattella germanica*) and either the oriental cockroach (*Blatta orientalis*) or the American cockroach (*Periplaneta americana*). Tests with ants should normally be performed with the Black garden ant (*Lasius niger)*.

Claim: flying insects. In case of an application for authorisation of a product with a claim of "killing flying insects" tests should be provided with flies, mosquitoes and wasps. Tests with flies should normally be performed with the house fly, *Musca domestica*. Tests with mosquitoes should normally be performed with *Culex* spp. Test with wasps should normally be performed with *Vespula* spp.

Claim: acaricide. If a product is claimed to be an acaricide tests should be provided with mites and ticks. What species should be used depends on the area of use (house dust mites in homes, flour mites in storage rooms, etc., for instance: *Dermatophagoides pteronyssinus, Tyrophagus putrescentiae, Acarus siro*). For mites and ticks relevant species can be found in sections 7 and 8.

Claim: other arthropods. For this claim the applicant should provide information on what organisms are relevant for the intended use. At least some example should be given and these should be tested.

Specific claim next to general claim:

Whenever efficacy against a specific organism is claimed next to a general claim or as specification of a general claim (e.g. crawling insects, including bedbugs), tests against this organism should be provided.

5.6.4.2.2.2 Laboratory tests and field trials

Test requirements for each test species can be found at the following sections dedicated to these insects/acarids. For other arthropods a field trial should be provided or a good justification why this is not appropriate.

5.6.4.2.3 Assessment of authorisation

5.6.4.2.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". For products with general claims the performance criteria per tested organism are the same as those for products with a specific claim for the test species. I.e. for crawling insects the criteria are the same as for cockroaches and ants, for flying insect the same as flies, mosquitoes and wasps, etc. The criteria can be found in the sections dedicated to these insects/acarids.

5.6.4.3 Cockroaches

5.6.4.3.1 Introduction

Cockroaches are a common and persistent problem in many households. These crawling insects (although several species can also fly) are scavengers allowing them to readily adapt to changing food availability. Cockroaches can carry bacteria such as Salmonella in areas co-inhabited by humans. Cockroaches are also identified as a major cause of allergies and asthma, particularly in children. Amongst the crawling insects, cockroaches are the most difficult to control.

The effect of biocides on these insects is mainly based on either contact, both dermal and tarsal, or the ingestion of bait products.

5.6.4.3.1.1 Biology

Cockroaches belong to the (sub) order Blattodea. There are over 3500 species of cockroaches, but only a few are considered domestic pests in the EU. The German cockroach, *Blattella germanica*, is the most common.

Upon hatching from an egg capsule, cockroaches begin their nymphal stage (smaller version of adults minus fully developed wings and sex reproduction organs) and moult through various instars until reaching the adult stage. Time of development can take weeks or months depending upon the species and the surrounding environmental conditions. For instance the eggs of German cockroaches hatch after 3 to 5 weeks (depending on the temperature), the nymphal stage (5 to 7 moultings) can be 40 days to 6 months and the adults live about 6 month (longer under lab conditions).

In temperate European countries most cockroach species will almost never be found outside, with foraging activities almost entirely within human-made structures.

5.6.4.3.2 Dossier requirements

A clear label with comprehensive claims should be submitted. The study results of trials should demonstrate the efficacy of the product based on the submitted label claim. Requirements can differ for products for professional use and for consumer products. For professional use a field trial is always required, for consumer products in some cases laboratory and simulated-use tests are sufficient. If the product is applied as a bait, the entire bait (formulated, including the bait box if applicable) should be tested, not only the active substance which is contained in the bait.

Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. Appendix 19 gives an example of a test guideline that can be used. If the available guidelines are not suitable, industry standard or a company's own protocols are acceptable, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. A control treatment without biocide (negative control) should be included in all laboratory trials.

In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factor that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, pest activity before the trial is initiated, general levels of sanitation, treatment history, season, etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.3.2.1 Test species

For use against cockroaches data against two key species, one small species normally German cockroach (*Blattella germanica*) and one large species either the Oriental cockroach (*Blatta orientalis*) or the American cockroach (*P. americana*), should normally be available for spray products (aerosol, space spray, residual spray) to support general claims against cockroaches. For bait products, the label can only claim efficacy against species that have been treated under field conditions.

5.6.4.3.2.2 Laboratory tests and field trials

For the evaluation of biocides against cockroaches different types of laboratory, simulated-use tests and field trials can be used. Examples of test are listed below.

Screening Studies (No- Choice Test)

The product is applied to representative surfaces or via direct cuticle application, in an arena with cockroaches, to assess inherent contact toxicity or knockdown effects of the active substance. Specify whether adults (male or female) or nymphs are used. Tests may be used to demonstrate basic efficacy or efficacy against insects, resistance to specific chemicals (LD50

versus a susceptible field strain) or insect growth regulator effects (nymphs are treated and subsequent effects are recorded such as inhibition of moulting, deformities, sterile adults).

Results support descriptions related to the mode of action (symptomology) or "effective against strains resistant to "x" class of insecticides", or similar efficacy claims.

For bait products dietary bioassay studies can be conducted using the biocidal bait as a food source. Replicate groups of test insects are exposed to either a continuous toxic diet, or a toxic diet for 24 hours and then a non-toxic diet for the rest of test period.

In all laboratory studies a treatment without biocide should be conducted as a negative control, with insects from the same insect population and with the same number of replicates.

Screening tests are not always necessary. When efficacy is demonstrated in residual tests, palatability tests or similar tests, this is deemed sufficient. Screening tests can sometimes be used as bridging studies: if tests involving a product result in similar effects in different target species, field studies can be waived for some insect species.

Determination of residual efficacy

Formulated product (spray, powder, dust, etc.) is applied to representative surfaces at a specified dose rate, or rates, including the recommended label rate(s). Cockroaches (adults) are exposed to the deposit at several time intervals after application (including the day of treatment and at the end of the claimed residual period). Exposure time should, preferably, be comparable to the time the cockroaches might reasonably be expected to be in contact with a treated surface under natural conditions (e.g. 10 min - 1 hour) and assessors will take this factor into consideration when evaluating the data. Treated surfaces should include at least one porous and one non-porous substrate (or according to the label claim) representing surfaces that might, typically, be treated for cockroach control (e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete). Mortality is normally assessed 1 day and up to 7 days post-exposure.

To substantiate a knockdown claim the number of cockroaches on their backs is counted at stated times after exposure (typically at 5 minute intervals until +30 min, then again at 45 and 60 min). The time until 50% (KT50) and 95% (KT95) of the insects are knocked down is derived statistically.

For insect growth regulators, exposure conditions can be as described above, but selection of the developmental stage (nymph, adult) and post-exposure assessment (deformities, moulting success, sterility, mortality) must be adapted to suit the mode of action of the active substance. Hence, assessments may continue to be made several weeks after exposure (sub-lethal or non-lethal effects on fertility, sterility for example may contribute to long term population control without short term mortality).

Groups of cockroaches of the target species should be of specified age/sex and number. Normally tests are performed with 5 or more replicates, with at least 10 cockroaches per replicate. When only 3 replicates are used, at least 20 insects per replicate should be used. Replicates should be conducted per applied dose, time point, surface, and a reference product (at registered rate) and untreated surfaces should be included as negative controls.

Environmental conditions must be specified for the test itself, and during storage of the treated substrates (temperature, humidity, photoperiod). Temperature would be expected to fall in the range 19-29°C. When efficacy at high temperatures is claimed 40°C would be a good test temperature.

Palatability tests with bait products

The aim of the bait choice feeding trials is to determine the palatability of the product for the test insect. If conducted on both fresh and aged product it may provide information on the storage stability of the product. In this test design, nymphs and adults of German and Oriental cockroaches have the choice between a non-toxic food source (challenge diet, either the non-toxic bait or a non-toxic food source known to be a strong feeding source for the test species) and the bait containing the active substance. Normally tests are performed with 5 or more replicate tests, with at least 10 cockroaches per replicate. When only 3 replicates are employed, at least 20 insects per replicate should be used. In all laboratory studies a treatment without biocide should be conducted with insects from the same insect population, as a negative control.

The test should demonstrate acceptable toxicity in competition with the alternative food source.

The population composition (males, gravid non-gravid females, nymphs) in these tests is of importance. Preferably mature insects should be used since immature stages do not need to feed every 24 hours. It should be noted that the feeding behaviour of German cockroach females, changes during 'pregnancy' and that early instar nymphs tend to forage less than older instars.

Simulated use

These tests are designed to mimic the practical use situation. The insects must have a choice to be in contact with the biocide or not. For example, cockroaches (*B. orientalis, B. germanica*) can be introduced into choice boxes with one half of the base surface being sprayed with a test formulation. Food and water is always on the non-treated area to be reached by the animals without crossing the treated area. Variations on this test would be to expose insects (voluntary contact) to a variety of different treated surfaces, e.g. plywood, cement, vinyl, ceramic tiles, glass etc.

For products claiming "population control" (eradicates cockroach population) an entire population or at least different life stages should be tested while there is a possibility that only a few individuals get in contact with the biocide.

For "secondary kill" (kills cockroaches that do not visit the bait, however, not always the whole population) claims at least different life stages should normally be tested where only a few individuals get in contact with the biocide directly. Life stage is dependent on a specific mode of action (necrophagy versus coprophagy) and the claim. Either nymphs or adults could be used.

In all laboratory studies a treatment without biocide should be conducted with insects from the same insect population, as a negative control.

Field trial

In field trials the product is tested in actual use situation, for instance in an infested home or warehouse and applied according to the direction for use on the label. An example of the results to be achieved in a field trial can be found in Appendix 19.

5.6.4.3.2.3 Requirements per type of claim

Per type of claim the requirements will be listed.

Products intended for use as general surface treatment or aerosol for consumers:

- a laboratory test showing mortality and/or knockdown and/or residual efficacy, depending on the claim;
- a simulated-use test showing mortality and knockdown according to the claim.

Products intended for use as general surface treatment or aerosol for professionals:

- a laboratory test showing mortality and/or knockdown and/or residual efficacy, depending on the claim;
- a simulated-use test showing mortality and knockdown according to the claim;
- a field trial according to the directions for use.

Products intended for use as general surface treatment or aerosol with a claim of population control or secondary kill:

- a laboratory test showing residual efficacy;
- a simulated-use test showing mortality according to the claim;
- a field trial according to the directions for use.

Products intended for use as baits:

- due to the specificity of baits, only effects against species of cockroach that have been tested in the field can be claimed on the product label;
- a laboratory test showing palatability, of fresh product and product at the end of the claimed maximum storage period;
- a simulated-use test showing mortality according to the claim;
- a field trial according to the directions for use and with the claimed cockroach species.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.3.3 Assessment of authorisation

5.6.4.3.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy" (BPD). This is implemented in the following way.

An insecticidal product intended for the control of cockroaches is normally considered to be sufficiently "effective" if the following results can be achieved:

Products intended for use as general surface treatment or aerosol for consumers:

- required results in laboratory tests and simulated-use tests:
  - ≥ 90% knockdown within a few minutes after contact with the product (or according to the claim), direct after spray and at the end of the residual period claimed;
  - mortality according to the label claim, preferably ≥90% in 24 hour.

Products intended for use as general surface treatment or aerosol for professionals:

- required results in laboratory tests:
  - direct application: 100% mortality within 1 hour after spraying the cockroaches, mortality between 90 and 100% can be accepted provided a qualified explanation is given for the lack of total control;
  - residual test: 100% mortality within 24 hours after placing the cockroaches in the test area, direct after spray and at the end of the claimed residual period. Mortality between 90 and 100% can be accepted provided a qualified explanation is given for the lack of total control.
- required results in field trial:
  - after a period of 2-10 weeks, the population reduction exceeds ≥90% relative to either untreated sites or pre-treatment levels. If retreatment is necessary 100% mortality should then be achieved.

Products intended for use as general surface treatment or aerosol with a claim of population control or secondary kill:

- required results in laboratory tests and simulated-use tests:
  - ≥ 90% mortality within the test period, direct after spray and at the end of the residual period claimed;
- required results in field trials:
  - after a period of 2-10 weeks, the population reduction exceeds 90% relative to either untreated sites or pre-treatment levels.

Products intended for use as baits:

- required results in laboratory palatability choice test (bait and alternative food):
  - at least 95% of the test insects have been killed at a given time point;
- required results in simulated-use tests:
  - ≥ 90% reduction of the population within a few weeks;
- required results in field trials:
  - after a period of 2-10 weeks, the population reduction exceeds 80% relative to either untreated sites or pre-treatment levels.

Products based in insect growth regulators (IGR):

- required results in laboratory tests:
  - at least 95% of the insects does not develop to the next instar;
- required results in simulated-use tests:
  - ≥ 90% reduction of the population within a few weeks;
- required results in field trials:
  - after a period of 6 -14 weeks, the population reduction exceeds 80% relative to either untreated sites or pre-treatment levels.

Deviation from these norms is possible but should be justified in the application.

Field trial data at the label application rate(s) must preferably be evaluated by an experienced assessor since performance can vary considerably, even from apartment to apartment in the same building. Number of trials, the complexity of the trials sites, the use (or not) of additional measures that can contribute to effective control, treatment history, etc. can all have a substantial effect upon the level of control that is achieved. The data must provide evidence of suitable levels of efficacy during the residual period claimed, relative to pre-treatment population assessments and/or performance of reference products under similar conditions, and/or assessments of cockroach populations in untreated areas under similar conditions. Where mean population reduction exceeds 90% relative to either untreated sites or pre-treatment levels, the product is considered effective, but the assessor has the discretion to view each data set on its merits and consider all factors before concluding whether the data support the claimed level of performance or not.

5.6.4.4 Ants

5.6.4.4.1 Introduction

Ants may cause inconvenience both indoors and outdoors.

In Europe the following ant species are common:

Black garden ant,	*Lasius* spp., most common *L. niger*
Pavement ant	*Tetramorium caespitum*
Red ant	*Myrmica rubra*
Erratic ant	*Tapinoma erraticum.*

Next to these native ant species tropical ants can cause inconvenience, mainly indoors.

Of the tropical ant species there are two species that are most commonly found causing inconvenience in buildings in Europe:

Pharaoh ant	*Monomorium pharaonis*
Argentine ant	*Linepithema humile.*

5.6.4.4.1.1 Biology

Ant development involves a complete metamorphosis that includes distinct egg, larval, pupal and adult stages. Most ant species form colonies comprised of complicated social structures that include infertile female workers, one or more specialised fertile queens and (at certain stages in nest development) sexually mature males. Some species have developed additional specialised workers that are responsible for guarding the nest and attacking intruders, whilst others perform domestic and foraging duties. These workers will actively forage on a wide range of foods including sweet substances, seeds, insects and aphid secretions. A successful foraging ant also has the ability to communicate where to find food to her co-workers, using chemical signals (trail pheromones).

5.6.4.4.2 Dossier requirements

A clear label claim should be submitted. The study results of field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Requirements can differ for products for professional use and for consumer products. For professional use products a field trial is always required, while laboratory and simulated-use tests might be considered sufficient in some cases for consumer products. Requirements also depend on the use: for "nest kill" and bait products alike, both laboratory and field trials with the test insects are needed; for products that only claim to kill individual insects that are in contact with the biocide, laboratory and simulated-use tests are sufficient. If the product is applied as a bait, the entire bait (formulated, including the bait box if applicable) should be tested, not only the active substance which is contained in the bait.

Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines.

If there are no guidelines available or the guidelines are not suitable, the applicant may use their own methods (intra-company Standard Operating Procedures), on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and

fully described and must address the efficacy claim that appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to the use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.4.2.1 Test species

Table 35 below shows for this group of insecticides the possible combinations of target organisms, and the corresponding test organisms on which efficacy is tested in both laboratory test and field trial. The selection of test species should be relevant to the label claim.

Table 35: Target organisms *versus* test organisms

Target organisms of the insecticide:	Test organisms:
Ants	Garden ant (*Lasius niger)*
Tropical ants	Pharaoh ant (*Monomorium pharaonis*), Argentine ant (*Linepithema humile*)

5.6.4.4.2.2 Laboratory tests and field trials

For the evaluation of biocides against ants different types of laboratory, simulated-use tests and field trials can be used.

Screening studies for direct spray or general surface treatments

In all laboratory studies a treatment without biocide should be conducted with insects from the same insect population, as a negative control. Examples of tests are:

Direct spray: 20 ants placed within a Petri dish and directly sprayed with material. Knockdown, time to death and total mortality is recorded. For insecticides with a "nest kill" claim the time to death will be longer (>1 day) since these ants have to live long enough to take the insecticide into the nest. Normally at least 5 replications and 5 non-treated controls should be used. Controls are very important in this case, as it often turns out to be very difficult to keep ants active in trials.

Residual spray: 20 ants placed on a surface treated with the product. Ants are placed in the arena directly after application, at several time intervals after application and also at the end of the period claimed for residual effect. The time to death of the ants and total mortality is recorded.

A control treatment without biocide should be included in all laboratory trials. Normally at least 5 replications and 5 non-treated controls should be used.

Palatability tests with bait products

The important factors relating to testing bait products are to establish the appropriate dosage and intrinsic palatability of the formulation in laboratory tests. Claims made for bait products should distinguish between ants and tropical ants, since the latter can be attracted by completely different baits than the more common European ant, *L. niger*. Data should be provided for all species, for which claims are made.

The most important factor involved in laboratory testing is to provide a free choice alternative food source to the test insects. This may be sugar-based materials for European ants and protein-based materials (meat, eggs, dead insects) for some tropical ants. The formulation should demonstrate acceptable toxicity in competition with the alternative food source. A control treatment without biocide of similar size as the test itself (i.e. number of replications) should be included in all laboratory trials.

When a product is claimed to be effective after a long period of storage, it is also necessary to demonstrate that the product will still be effective, and attractive, after the stated storage period. The applicant must either provide data on the palatability of the product at the end of maximum storage period or alternatively (in case of a new product) data gained in a stress test

with 'accelerated ageing', i.e. a palatability test with the product which is stored under challenging conditions.

Simulated-use tests

These tests are designed to mimic the practical use situation. The tests should be relevant to the use and label claims. A control treatment without biocide should be included in all laboratory tests. Control trials should be of similar size (i.e. number of replications) as the test itself, to make statistical comparison possible and to get a fair impression of control mortality.

Examples of tests are:

Direct general surface treatments without nest kill:
Ants (normally at least 20 worker ants) can be introduced into choice boxes/arenas with one half of the base surface being sprayed with a test formulation, at the correct application rate according to the product label. Food and water is always on the non-treated area to be reached by the animals without crossing the treated area. Variations on this test would be to expose insects (voluntary contact) to a variety of different treated surfaces, e.g. plywood, cement, vinyl, ceramic tiles, glass etc. Mortality is recorded.

Normally tests should be performed in triplicate.

Direct general surface treatments with nest kill:
In a double chamber trial an ant's nest (normally at least 20 (worker) ants) is placed within one arena, which is connected to another arena. Part of the second arena is treated with the insecticide at the correct application rate according to the product label. Adequate food and water is placed on the non-treated surface of this second arena. Ants must be able to reach the food without contacting the treated surface. Normally tests should be performed in triplicate. Efficacy is assessed e.g. length of time taken to result in control of the ant population (e.g. no foraging ants).

The nest should be opened at the end of the trial (e.g. 1 week), to check whether all ants within the nest are dead, especially the queen(s).

Bait products:
The efficacy of the entire formulated bait is tested, hence not only the active component within the bait. An ant's nest is placed within an arena trial under controlled conditions (e.g. with respect to temperature, relative humidity, photoperiod, etc.). Adequate food (bait without the active substance or and alternative food source) and water are placed opposite the nest. Insects are allowed to acclimatise for 7 days before introduction of bait. An additional fasting period of 4 days, providing them with water only, is recommended. At regular time intervals (in hours), the attractiveness of the bait for the ants is recorded (by observing whether they approach the bait or avoid it). Ant mortality is recorded at regular time intervals (in days). At the end of the trial the nest could be opened to check whether all ants within the nest, including the queen(s), are dead.

Field trials for all claims

The tests should be relevant to the use and label claims. Tests with *Lasius niger* are done preferably during the early spring. In the end of summer population decline might be due to natural causes instead of the insecticide. Non-treated nests should be used as a negative control, to test nest activity.

Monitor ant numbers at various locations around a building and locate the entrances of nests and "ant-trails" (routes taken by ants). Apply the insecticide according to the label instructions.

The efficacy tests against ants should normally be performed in a minimum of three objects. An object can be a place in or near the house, where ants cause inconvenience for the inhabitants. This may be in a house, on a balcony, a terrace or in a garden, depending on the field of use of the product. If the test is performed outdoors, records of temperature and rainfall should be kept.

Monitoring should be conducted at the same locations (as the pre-treatment) and at similar times during the entire trial (e.g. at 12.30, 13.00, etc.). Monitoring should continue (e.g. 1 day after treatment, 1 week after treatment, etc. at least once weekly) until control is seen. If no ants are seen during a post-treatment monitoring visit then the site should be re-visited once to ensure that re-infestation does not occur.

The effect on the ant population can be determined by counting. For this purpose, a fixed position on the 'ant-trail' is to be used and a count of the number of any ants that pass is made in 1 minute, at several time intervals during the test.

5.6.4.4.2.3 Requirements per type of claim

Per type of claim the requirements will be listed.

Products intended for use as general surface treatment for consumers:

- a laboratory test showing mortality and/or knockdown and/or residual efficacy, depending on the claim;
- a simulated-use test showing mortality and knockdown according to the claim.

Products intended for use as general surface treatment for professionals:

- a laboratory test showing mortality and/or knockdown and/or residual efficacy, depending on the claim;
- a simulated-use test showing mortality and knockdown;
- a field trial according to the directions for use.

Products intended for use as general surface treatment with a claim of nest kill:

- a laboratory test showing residual efficacy;
- a simulated-use test showing mortality;
- a field trial according to the directions for use.

Products intended for use as baits:

a) Due to the specificity of baits, only effects against ant species that have been tested in the field can be claimed on the product label;
b) a laboratory test showing palatability;
c) a simulated-use test showing mortality;
d) a field trial according to the directions for use and with the claimed ant species.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.4.3 Assessment of authorisation

5.6.4.4.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy" (BPD). This is implemented for ants in the following way.

An insecticide against ants is normally considered to be sufficiently "effective" if the following results can be achieved:

Products intended for use as general surface treatment for consumers:

- required results in laboratory mortality tests and simulated-use tests:
  - ≥ 90% knockdown in 5 -10 minutes (or according to the claim), direct after spraying the ants and at the end of the residual period;
  - mortality according to the label claim, preferably ≥90% after 24 hour.

Products intended for use as general surface treatment for professionals:

- required results in laboratory tests:
  - direct application: 100% mortality within 24 hours after spraying the ants, mortality between 90 and 100% can be accepted provided a qualified explanation is given for the lack of total control;
  - residual tests: ≥ 90% mortality within 24 hours after placing the ants in the test area, direct after spray and at the end of the residual period;
- required results in field trials:
  - after a period of 2-8 weeks, the population reduction exceeds 90% relative to either untreated sites or pre-treatment levels.

Products intended for use as general surface treatment with a claim of nest kill:

- laboratory tests:
  - 100% mortality within the test period, direct after spray and at the end of the

residual period;

- required results in simulated-use tests:
  - slow knockdown, ants must be able to reach the nest;
  - ≥ 90% mortality within the test period, including ants in the nest;
- required results in field trials:
  - after a period of 2-8 weeks, the population reduction 100% relative to either untreated sites or pre-treatment levels, in case of lower efficacy it has to be shown that the queen(s) in the test nests is killed.

Products intended for use as baits:

- required results in laboratory palatability choice test (bait and alternative food):
  - at least 95% of the test insects have been killed at a given time point;
- required results in simulated-use tests:
  - ≥ 90% reduction of the population within a few weeks;
- required results in field trials:
  - after a period of 2-4 weeks, the population reduction exceeds 90% relative to either untreated sites or pre-treatment levels.

Deviations from these norms is possible but should be justified in the application.

5.6.4.5 Termites

5.6.4.5.1 Introduction

Termites, in natural settings, work as beneficial insects by breaking down cellulose-containing materials, such as dead trees. However, termites can cause damage to living trees and many crop plants, but the fact that they can use dead wood makes them a major pest for timber used both outdoors and inside buildings. Termites become a problem to humans when they infest timber used in constructions (i.e. wood structures) in risk areas. Owing to their high moisture requirements, they usually nest in soils, but can invade buildings from underneath through cracks and seams or by building shelter tubes connecting the wood to their nest in the soil. In Europe and in the European tropical overseas regions, there are three main types of termites: subterranean, tree and drywood termites, the subterranean being the most destructive termites in construction. Due to their biological characteristics (subterranean termites), they live in the soil and must maintain contact with the ground or some other moisture source to survive.

Insecticides against termites can be divided into PT8 products, preventive treatments to protect the wood and curative treatments on the wood, and PT18 products, which are considered in this section.

5.6.4.5.1.1 Biology

Termites belong to the order of Isoptera. In Europe and in the European tropical overseas regions there are three main termite families; subterranean (*Rhinotermitidae*), drywood termites (*Kalotermitidae*) and tree termites (*Nasutitermitidae*).

Reticulitermes is the most common genus encountered from the Rhinotermitidae family in Europe. The main species registered are: *R. flavipes (former R. santonensis), R. lucifugus, R. lucifugus corsicus, R. grassei, R. banyulensis, R. balkanensis*.

They are widespread around the Mediterranean (Spain, France, Italy, Portugal, Balkans, and Greece) and Black Sea (Turkey, Rumania), though some termite spots in the UK and Germany have been reported. Several unanswered questions remain about the origin of these termites. While some Reticulitermes are native to Europe, others may be related to species from eastern North America and the Middle East (Israel, Asian Turkey, etc.).

Coptotermes sp. and *Heterotermes sp.* are the main two species belonging to the *Rhinotermitidae* family found in European tropical overseas regions.

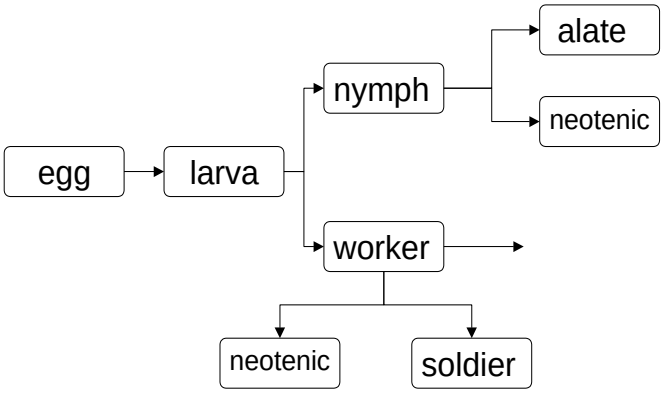
Nasutitermes sp. are the main species belonging to the *Termitidae* family (tree termites) encountered in the European tropical overseas regions.

Kalotermes flavicollis and *Cryptotermes brevis* are the main two species of drywood termites present in Europe (especially in the coastal areas of Mediterranean countries and Canary

Islands). *Cryptotermes sp*. is a main genus belonging to drywood termites encountered in the European tropical overseas regions.

A brief explanation of the life cycle (Figure 11) may help to clarify the difficulties involved in control of termites. There is a split after the larval stages into two lines, the sexual and the worker line. Individuals going down the sexual line develop into nymphs and then into either alates (which are the reproductive form most people are familiar with) or neotenics (supplementary reproductives). The alates do form queens (physogastrics), however, these are much more mobile than those found in tropical species. The alternative line of development, the neutral line, is the development of larvae into workers, which in turn can either remain workers or develop into neotenics or soldiers. Workers are approximately 4 to 6 mm in length. An important feature in the biology of termites that makes them very difficult to control is the ability of individuals in both lines to form sexual reproductives and, hence, give rise to a new, viable colony. In addition, supplementary secondary reproductives can be produced in very large numbers.

Figure 11: Life cycle of subterranean termites



5.6.4.5.1.2 Control methods

Preventive treatments

Traditionally, the methods used to fight termites were based upon treating infested or exposed wood with wood preservatives. This is valid for all termite types (subterranean, tree and drywood). Those products are included in product type 8 (wood preservatives) of the BPD, and are not considered in this section.

In addition to the preventive treatment of timber, a barrier can be used to isolate the paths used by subterranean termites to access the building from underneath where the nest is located. Barriers systems usually consist of a polymer membrane or other material and an insecticide (product type 18). The system is installed between the soil and the construction to keep subterranean termites outside and to eliminate those that come into contact with the insecticide.

Remedial treatments

Different methods are currently used in Europe:

Chemical barriers
Methods based on treating the infested wood with wood preservatives are included in product type 8 (wood preservative) of the BPD, and are not considered in this section.

In addition to the wood treatment, two types of chemical barriers are used to impregnate the walls of the construction and the soil around.

Considering the subterranean termites, this method aims to eliminate insects inside the construction and to protect it for several years. This method does not eliminate the nest (which is located in the soil).

Bait system
It consists typically of a cellulose-based matrix treated with a slow acting insecticide, which is consumed by workers and is spread through the colony by trophallaxis (one individual is fed by another). Consequently, this method may be useful to eradicate the whole colony.

Treatment of waste

In order to prevent termite contamination by waste infested and transported into an area not infested, it could be relevant to treat the waste with biocidal products.

5.6.4.5.2 Dossier requirements

A clear label claim should be submitted.

Laboratory and field trials with termites are needed to assess the efficacy of the products. Ideally, the studies should be performed according to established guidelines where these are available. These may be EU or national guidelines. European standardisation work is being conducted by several termite experts in Europe. At this moment, no European standard has been published yet, only French standards are available. However, due to the greater significance of termites as structural pests in countries outside Europe, such as the United States and Australia, a variety of standard test methods are published, together with extensive reports in the scientific literature which may prove useful references. Account should be taken of results obtained using such methods, especially where the same termite species are present as those in Europe including the French overseas territories. See Appendix 18 for a list of available guidelines (guidelines outside EU not included yet).

If there are no guidelines available or guidelines are not suitable to evaluate the termiticide (e.g. if new products are developed), the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. In the case of field trials a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, treatment history, etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

A control treatment without biocide should be included when testing any termite products in laboratory trials.

5.6.4.5.2.1 Test species

A product against termites in Europe should normally be tested on termites belonging to the genus Reticulitermes.

For European tropical overseas regions, the product should normally be tested at least against termites belonging to the genus *Coptotermes* and on every genus claimed by the applicant.

Remarks:

e) In any case, the termite species needs to be identified and all useful information about the colony collected (locality of origin, laboratory rearing conditions, characteristics of their natural environment if termites are collected in field);

f) For the evaluation of termite baits, the species referred to in the label claims should be used. If the claim refers generally to Reticulitermes species (without specifying the species), it is recommended to test, at least, two different European species in lab tests;

g) Due to the specificity of baits, only effects against species of termites that have been tested should be claimed on the product label.

5.6.4.5.2.2 Laboratory tests and field trials

The tests specified below are mainly for bait products. While laboratory tests can be conducted for all the termiticide products, field trials are addressed specially for bait products. For soil/wall barrier products and for physico-chemical systems the tests should be designed to mimic the practical use situation. The test should be performed according to the label claim.

Due to the specificity of baits, only effects against species of termites that have been tested should be claimed on the product label.

The important factors relating to testing bait products are to:

a) establish the appropriate dosage of the formulation in laboratory tests. This can be done in a mortality test (evaluation of the toxicity of the insecticidal formulation in a force-feed environment). The formulation should demonstrate acceptable toxicity;

b) test the palatability of the bait. The aim of the bait choice feeding trials is to determine the palatability of the product for the test insect. In this test design, insects have the choice between a non-poisoned food source (challenge diet) and the bait containing the active substance;

c) the test should demonstrate acceptable toxicity in competition with the alternative food source;

d) assess if a contaminated group of termites can transfer the insecticide to a group of termites that have never been exposed to it before. This transfer study should demonstrate acceptable toxicity of termites not exposed directly to the baits.

Laboratory/screening tests

No-choice test (A): test the termiticidal efficacy and the delayed effect of an insecticide formulation on a group of subterranean termites":

A group of termites is put into contact with an insecticide formulation. When testing baits, bait is the only source of food. For other types of termiticides the termites are exposed to the product according to the intended use (e.g. spray the surface and add the termites to the surface. The test is performed in assay containers. Mortality of the insects is assessed.

From this test the time "te" can be determined, necessary to perform the test B (te=time of exposure of the termites to the insecticide formulation which is required to observe a significant mortality compared with termites in an untreated control).

Transfer test (B): the transmission of the insecticide used in the baiting system to an uninfected group of termites:

Termites are exposed to the tested bait long enough to be contaminated with the active substance (time te). A group of termites is removed from the colony and put in contact with a healthy uncontaminated group. The mortality rate of both groups of termites (contaminated and uncontaminated) is assessed separately.

Choice test / palatability test (C): the suppression of a group of termites reared in laboratory under conditions of food competition; with the use of the same insecticidal bait formulation:

Add the insecticidal bait formulation to a group of termites already exploiting another source of food. The test is performed in assay containers. The aim is to assess the mortality after a given period of time.

Field trial

In field trials the product is tested in actual use situation and applied according to the direction for use on the label. The test method should evaluate the efficacy of the baits or barrier products in an experimental site where termite activity is reported.

The repellent termite barriers can be disposed in walls or soils, according to the claim. A common claim for a barrier product is the duration of "protection". This is normally in terms of a number of years and should be demonstrated by long-duration soil tests in field plots.

For bait products consumption of the tested bait must be registered at least in the first 6 months after the introduction of the baits. The elimination of termites in the experimental site should be registered maximum after 18 months (counted since the introduction of the first tested bait), excluding the winter period.

Table 36 gives an overview of available (French) guidelines for termites and how to use them.

Table 36: Overview guidelines on termites

Preventive treatment/Physico-chemical barrier		
	Protocol	**Ageing Test**
Laboratory test	NF X 41-550 after after after	 NF X 41-568 (effect of water) CTBA-BIO-E-016 (effect of the natural light) CTBA-BIO-E-007 (effect of alkalinity)
Field trial	CTBA-BIO-E-008	No
Remedial treatment/chemical barrier		
	Protocol	**Ageing Test**
Laboratory test	NF X 41-550 after	 NF X 41-542 (effect of water)
Field trial Wall chemical barrier Soil chemical barrier	NF X 41-550 after	 FCBA-BIO-E-053
Remedial treatment/Bait system		
	Protocol	**Ageing Test**
Laboratory test	XP X 41-543-1	No
Field trial	XP X 41-543-2	No

5.6.4.5.3 Assessment of authorisation

5.6.4.5.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy" (BPD). This is implemented in the following way.

An insecticidal product intended for the control of termites is normally considered to be sufficiently "effective" if the following results can be achieved (derived from standards NF XPX41-551, NF XPX41-543-3 and FCBA-BIO-E-041):

Products intended for use as baits:

- no-choice test: 100% mortality before the end of the test (16 weeks). Besides, if the 100% mortality is achieved too fast (less than 48 hours) the test bait should be rejected;
- transfer test: 100% mortality of all the termites, which have not been exposed directly with the tested bait;
- choice test / palatability test: more than 95% mortality;
- bait field trial: No termite activity should be reported within the test period (max. 18 months, excluding the winter period). No termite activity should be reported in at least the following 3 months.

Products intended for use as termite barriers

- laboratory test: 100% mortality after the test (only for barriers with lethal activity);
- field trial:
    - In soil barrier products, termites should not penetrate the soil more than 10 mm;
    - In wall barriers (i.e. thermoplastic films), termites should not be able to perforate the film after the duration of the test;
    - In other types of repellent barriers, termites should not be able to access the other side of the barrier. Furthermore, any carrying of termite material (i.e. soil) to the other side of the barrier should not be reported.

5.6.4.6 Bed Bugs

5.6.4.6.1 Introduction

Bedbugs are small, wingless blood feeding insects. Of the many recognized species, only three are known to feed on humans. In temperate climate regions of the EU, *Cimex lectularius* is the dominant species. Bedbugs are not known to transmit disease in Europe, but infestations can cause painful and irritating bites on the skin while humans sleep. Once infested, treatment and control is very difficult.

A sign of bedbug presence include bites on the exposed skin (small red itchy bumps) of humans during sleep. If observed, confined locations such as mattress linings or furniture folds should be inspected for faecal spotting and the presence of bedbugs.

5.6.4.6.1.1 Biology

Bedbugs belong to the order of Hemiptera, Family Cimicidae.

Bedbugs harbour themselves in very confined areas in wall cracks, furniture joints, along lining of mattresses, behind pictures and in seams of furnishings. These insects generally confine themselves to these areas and leave them only to feed. Bedbugs are negatively phototactic and not usually seen outside the harbourage in the day or when the lights are on.

Female bedbugs can lay up to 500 eggs during their lifetime. Depending on frequency of blood meals, bedbugs can live for more than a year. They are able to survive for months without feeding (dependent upon temperature: at 16°C survival can be a year). The first nymph hatch from small white eggs after 7-10 days at room temperature (around 20°C) and earlier at higher temperatures. Each of the 5 nymphal stages need a blood meal to complete development to the next instar. The whole life-cycle from egg to egg takes a minimum of 28 days at 27°C or around 42 days at 22°C.

5.6.4.6.2 Dossier requirements

A clear label claim should be submitted. The study results of field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Laboratory and field trials with bedbugs are needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines.

If there are no guidelines available or guidelines are not suitable, the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.6.2.1 Test species

A product against bedbugs should normally be tested on the common bedbug (*Cimex lectularius*) or tropical bedbug (*Cimex hemipterus*).

5.6.4.6.2.2 Laboratory tests and field trials

For the evaluation of biocides against bedbugs different types of laboratory, simulated-use tests and field trial can be used. Examples of tests are listed below.

Screening studies (no- choice test)

Testing should include application of the product to representative surfaces (e.g. plywood, painted plywood, textile fabric, wallpaper) or direct cuticle application of the product to bedbugs to assess inherent contact toxicity of the active substance. It should be specified whether adults or nymphs are used. A test may be used to demonstrate basic efficacy or efficacy against insects resistant to specific chemicals (LD50 versus a susceptible field or

laboratory strain) or insect growth regulator effects (nymphs are treated and subsequent effects are recorded such as inhibition of moulting, deformities, sterile adults).

Results must support description related to the mode of action (symptomology) or "effective against strains resistant to "x" class of insecticides", or similar efficacy claims.

Screening tests are not always necessary. It is sufficient to demonstrated efficacy in residual tests or similar tests.

Determination of residual efficacy

Good residual efficacy is essential for insecticides used in bedbug control, as is impossible to treat all bedbugs directly or reach all of their hiding.

For the determination of residual efficacy, the formulated product (spray, powder, dust, etc.) should be applied to representative surfaces at the recommended label rate. Bedbugs (adults) should be exposed to the deposit at several time intervals after the deposit has dried (including the day of treatment, but after the deposit has dried completely and at the end of the claimed residual period). Exposure time should, preferably, be comparable to the time the bedbugs might reasonably be expected to be in contact with a treated surface under practical conditions (e.g. 10 min - 6 hours) and assessors will take this factor into consideration when evaluating the data. Treated surfaces should include at least two porous and one non-porous substrate, representing surfaces that might, typically, be treated for bedbug control (e.g. plywood, painted plywood, textile fabric, wallpaper, according to the label claim). Mortality is normally assessed after 1 day up to 14 days post-exposure.

For insect growth regulators, exposure conditions can be as described above, but selection of the developmental stage (nymph, adult) and post-exposure assessment (deformities, moulting success, sterility, mortality) must be adapted to suit the mode of action of the active substance. Hence, assessments may continue to be made several weeks after exposure (sub-lethal or non-lethal effects on fertility, sterility for example may contribute to long term population control without short term mortality).

Groups of bedbugs should be of specified age/sex and number. Tests should be performed in triplicate, with at least 20 bedbugs per replicate. When 5 or more replicates are used, 10 insects per replicate are adequate. Replicates should preferably be conducted per applied dose, time point, and surface. Untreated surfaces must be included as negative controls.

Environmental conditions must be specified for the test itself, and during storage of the treated substrates (temperature, humidity, photoperiod). Temperature would be expected to fall in the range 19-29°C. For use in Southern European countries higher temperatures (up to 40°C) might be necessary.

A control treatment without biocide should be included in all laboratory trials. The control trial should be of adequate size (i.e. number of replications and individuals), providing sufficient statistical power and a fair impression of control mortality.

Simulated use

These tests are designed to mimic the practical use situation. The insects must have a choice to be in contact with the biocide or not. Due to the normal behaviour of the bedbugs, it seems to be very difficult to design simulated-use tests for the evaluation of products for bedbug control. Bedbugs do not leave their harbourage during daytime and without a host which attracts them.

Field trials

In field trials the product is tested in actual use situations, for instance in an infested home or hotel and applied according to the direction for use on the label.

It has to be considered that in bedbug infestations the aim of professional control operations must be the eradication of the population. It is not acceptable to have even very small remaining populations. Usually, pest control operations against bedbugs have to combine different measures. The documentation of the trial has to give all information on the products or other measures used.

5.6.4.6.2.3 Requirements per type of claim

Appropriate efficacy tests are needed for each claim.

Products intended for use as general surface treatment for consumers:

- a laboratory test showing mortality and/or knockdown and/or residual efficacy, depending on the claim.

Products intended for use as general surface treatment for professionals:

- a laboratory test showing mortality and/or knockdown and/or residual efficacy, depending on the claim;
- a simulated-use test showing mortality and knockdown according to the claim and/or;
- a field trial according to the directions for use;
- Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.6.3 Assessment of authorisation

5.6.4.6.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy" (BPD). This is implemented in the following way.

An insecticidal product intended for the control of bedbugs is considered to be sufficiently "effective" if the following results are achieved:

Products intended for use as general surface treatment for consumers:

- required results in laboratory tests (and simulated-use tests):
  - ≥ 90% knockdown within a few minutes after contact with the product (or according to the claim), direct after application and at the end of the residual period;
  - mortality according to the label claim, preferably ≥90% in 1 hour.

Products intended for use as general surface treatment for professionals:

- required results in laboratory tests:
  - direct application: 100% mortality within 24 hours after spraying the bedbugs;
  - residual test: ≥ 95% mortality within 24 hours after placing the bedbugs in the test area, direct after spray and at the end of the residual period.
- required results in field trial:
  - after a period of 6-10 weeks, the population reduction exceeds 90% relative to either untreated sites or pre-treatment levels.

    Treatment repeats usually are necessary in bedbug control. At the end of a treatment, 100 % efficacy should be achieved.

Deviations from these norms is possible but should be justified in the application.

Data from field trials at the label application rate must preferably be evaluated by an experienced assessor since performance can vary considerably, even from apartment to apartment in the same building. The number of trials, the complexity of the trials sites, the use (or not) of additional measures that can contribute to effective control, treatment history etc. can all have a substantial effect upon the level of control that is achieved. The data must provide evidence of suitable levels of efficacy during the residual period claimed, relative to pre-treatment population assessments and/or performance of reference products under similar conditions, and/or assessments of bedbug populations in untreated areas under similar conditions. Where mean population reduction exceeds 90% relative to either untreated sites or pre-treatment levels, the product is considered effective, but the assessor has the discretion to view each data set on its merits and consider all the factors before concluding whether the data support the claimed level of performance.

5.6.4.7 Ticks

5.6.4.7.1 Introduction

Ticks are small arthropods classed along with mites and spiders in the Class Arachnida. All ticks are blood feeders. Certain tick species are known for carrying and transmitting many different pathogenic micro-organisms including bacteria, viruses, parasites and fungi. Diseases associated with tick transmission in Europe include Lyme disease, tick-borne encephalitis, and

human anaplasmosis, all transmitted by *Ixodes ricinus*. The tick *Hyalomma marginatum* can transmit Crimean-Congo haemorrhagic fever, a viral disease common in East and West Africa. Mediterranean spotted fever is transmitted by the brown dog tick (*Rhipicephalus sanguineus*). Ticks also have an important role in animal health. They can cause anaemia, reduction of milk production and bodyweight gain of animals.

5.6.4.7.1.1 Biology

Ticks differ from insects morphologically having two main body parts (insects have three) and eight legs as nymphs and adults (six legs for insects). Ticks go through four stages to complete their lifecycle: egg, larva, nymph, and adult. Feeding will occur in both the immature and adult stages. After mating female hard ticks will feed once more followed by oviposition of hundreds to even thousands of eggs.

Ticks can be differentiated on their host choices:

- one host: developing stages and adults feed on one host (e.g. *Boophilus*)*;*
- two hosts: larvae and nymphs feed on the same host, adults feed on another host (e.g. *Rhipicephalus)*;
- three hosts: larvae, nymphs and adults feed on three different hosts. *(e.g. Ixodes, Haemophysalis, Dermacentor*)*.*

Ticks can be classified into two main families: soft ticks (Argasidae) and hard ticks (Ixodidae). The hard ticks consist of many commonly known species such as the sheep tick (*Ixodes ricinus*), the brown dog tick (*R. sanguineus*) and *Dermacentor sp* . *H. marginatum* is also a hard tick. Hard ticks vary in host-tick relationship. Species may have one host, two different hosts or three different hosts. After mating female hard ticks will feed once more followed by oviposition of hundreds to even thousands of eggs.

Soft ticks have similar body parts as the hard ticks. Key differences are that soft ticks lack the sclerotized outer cuticle found in hard ticks and the mouthparts of soft ticks are located below the end of the body (hard tick mouthparts stick out the front of the protected hood). For example the bird ticks, *Argas reflexus* and *A. persicus*, are soft ticks which can be a pest in for instance poultry farms.

Hard ticks have to be fixed to their hosts and the meal can last five days, while soft ticks are not fixed and the meal is finished in 20 to 50 minutes.

When searching for a possible host, ticks generally remain stationary until a host passes by. Once attached, ticks crawl to locate a place to feed. Commonly, ticks will attach to human skin along pant or sock lines or other tight locations which are warm and humid. Feeding can take hours to days depending on the species.

The bird ticks, *Argas persicus* and *A. reflexus* have worldwide distribution in warm climates. *A. persicus* occurs in small poultry farms and feeds blood on chicken and other domestic fowls. *A. reflexus* occurs in pigeon farms and on urban pigeons and their surroundings in towns. They can get from the nests of pigeons to lofts and attic rooms and feed on sleeping humans for blood. *A. reflexus* is an urban pest parasitizing urban pigeons and may cause a wide range of allergic reactions.

Argas spp. hide in cracks and crevices of chicken houses, nests, wooden equipments etc. during the day and come out to blood feed at night. Males and females are both blood sucking. They are able to survive starvation for two years, which is why the protection against these mites is very difficult.

5.6.4.7.2 Dossier requirements

A clear label claim should be submitted. The study results of field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Laboratory and, for some claims, field trials with ticks are needed to assess the efficacy of the product. The studies should normally be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If no guidelines are available, the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that

appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single acaricidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.7.2.1 Test species

A product against ticks should normally be tested on the sheep tick *Ixodes ricinus*. When control of dog or bird ticks is claimed, tests with these ticks should be performed too (*Rhipicephalus sanguineus*, *A. reflexus*). When efficacy in the tropics is claimed or efficacy against *H. marginatum*, this tick should be tested too. *H. marginatum* behaves differently than *I. ricinus* since it is aggressive and it actively seeks the host to feed on and moves quickly on the ground. When the product is intended for use in poultry farms tests should be performed against *A. persicus*.

5.6.4.7.2.2 Laboratory tests and field trials

For the evaluation of biocides against ticks different types of laboratory and simulated-use tests can be used. Examples of tests are listed below.

Laboratory test to evaluate knockdown and kill effect (no-choice test)

A clear label claim should be submitted. The study results of field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Laboratory and, for some claims, field trials with ticks are needed to assess the efficacy of the product. The studies should normally be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If no guidelines are available, the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single acaricidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

Simulated-use tests

To prevent disease transmission ticks must be knocked down, or killed before attaching to the skin. For products that knockdown and kill ticks a simulated-use tests should be performed in which the product is applied according to the instruction for use and then tested in the presence of a person or an arm or foot or animal. For some products this can be a similar test set up as described in 5.6.4.7.2.2. Then it has to be established that the ticks are knocked down or killed before they can attach to the skin and start feeding. This is compared to a control test.

5.6.4.7.2.3 Requirements per type of claim

Insecticide with knockdown or kill effect: laboratory and simulated-use tests.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.7.3 Assessment of authorisation

5.6.4.7.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy" (BPD). This is implemented for ticks in the following way.

An insecticide against ticks is normally considered to be sufficiently "effective" if the following results can be achieved:

- Product with knockdown effect:
  - 100% knockdown before ticks start feeding and;

- ≥ 80% kill within 24 hours;

- Product with kill effect:
  - ≥ 95% kill before ticks start feeding.

Deviations from these norms is possible but should be justified in the application.

5.6.4.8 Mites

5.6.4.8.1 Introduction

Mites, along with ticks, belong to the subclass Acarina (also known as Acari) and the class Arachnida. Mites are among the most diverse and successful of all the invertebrate groups. They have exploited an incredible array of habitats, and because of their small size (most are microscopic) most go totally unnoticed. Perhaps the best-known mite, is the house dust mite (family Pyroglyphidae), which can cause asthma and allergic symptoms. Mites are also important as vectors of micro-organisms, transmitting rickettsiae and bartonellae. Flour mites (*Acarus siro*) and mould or storage mites (*Tyrophagus putrescentiae, T. longior*) are important pests in stored goods. Mites like the red mite, *Dermanyssus gallinae*, can be a pest in bird cages and poultry farms. The red mite can also feed on some species of mammals, including humans, but need an avian host to reproduce.

Part of the control of mites is covered in section 5.6.4.11 on stored goods. Often mites are only mentioned on a label as a secondary pest, while insects are the main pests.

5.6.4.8.1.1 Biology

The house dust mite is widespread in human habitation. House dust mites thrive in the indoor environment provided by homes, specifically in bedrooms and kitchens. Dust mites survive well in mattresses, carpets, furniture and bedding, with figures around 188 animals/g dust. Dust mites feed on organic detritus such as flakes of shed human skin and flourish in the stable environment of dwellings. The European house dust mite (*Dermatophagoides pteronyssinus*) and the American house dust mite (*Dermatophagoides farinae*) are two different species, but are not necessarily confined to Europe or North America; a third species *Euroglyphus maynei* also occurs widely. The average life cycle for a male house dust mite is 10 to 19 days. A mated female house dust mite can live for 70 days, laying 60 to 100 eggs in the last 5 weeks of her life.

The flour mite, *A. siro*, is the most common species of mite in foodstuffs. The males are 0.33 mm to 0.43 mm long and female are 0.36 mm to 0.66 mm in length. Flour mites contaminate grain and flour by allergens and they transfer pathogenic micro-organisms. Foodstuffs acquire a sickly sweet smell and an unpalatable taste. When fed infested foodstuff, animals show reduced feed intake, diarrhoea, inflammation of the small intestine and impaired growth.

The red mite, *Dermanyssus gallinae,* is an ectoparasite of poultry and birds. They can be found in houses of laying hens, chickens and other fowls. The mites are blood feeders and attack resting birds at night. The optimal temperature is 27-28 °C. After feeding they hide in cracks and crevices away from daylight, where they mate and lay about 30-35 eggs in their lifetime. Their maximal lifetime is 8 weeks without starving and 6-10 months with starving. In spite of that these mites are ectoparasites, the main method of control is treating of the walls, bird cages, nests and hidden places in poultry farms with biocides.

5.6.4.8.2 Dossier requirements

A clear label claim should be submitted. The study results of laboratory or field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Laboratory and/or field trials with mites are needed to assess the efficacy of the product. The studies should normally be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If no guidelines are available or guidelines are not suitable, the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods might not be restricted to use of a single acaricidal product, a full description of any factors that might be expected to

influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.8.2.1 Test species

Which test species should be used depends on the intended area of use and the label claim. In homes the European house dust mites, *D. pteronyssinus,* is the most important. In storage rooms the flour mite or storage mites, etc., for instance *T. putrescentiae, A. siro*. For use on poultry farms *D. gallinae* should be tested. When specific mite species are mentioned in the claim these should be tested.

5.6.4.8.2.2 Laboratory tests and field trials

For the evaluation of biocides against mites different types of laboratory and simulated-use tests can be used. Examples are given below.

Laboratory test to evaluate knockdown and kill effect (no-choice test)

The product is applied to representative surfaces or via direct cuticle application, in a container with mites, to assess inherent contact toxicity or knockdown effect of the active substance. For instance spray on a filter paper and put the filter paper in an aluminium dish. Specify whether adults (male or female) or nymphs are used. Normally tests are performed with 3 or more replicates, with normally 20 to 30 mites per replicate. Tests are done at 25°C and 70-75% R.H.. In all laboratory studies a treatment without biocide should be conducted with mites from the same population, as a negative control. The number of dead mites is counted at 24 hours after treatment.

Residual effect

For determination of residual efficacy, the formulated product should be applied to representative surfaces at a specified dose rate, or rates, including the recommended label rate. Mites should be exposed to the deposit at several time intervals after the deposit has dried (including the day of treatment, but after the deposit has dried completely and at the end of the claimed residual period). Exposure time should, preferably, be comparable to the time the mites might reasonably be expected to be in contact with a treated surface under practical conditions and assessors will take this factor into consideration when evaluating the data. Treated surfaces should include at least two porous and one non-porous substrate, representing surfaces that might, typically, be treated for mite control (e.g. plywood, painted plywood, textile fabric, according to the label claim). Mortality is normally assessed after 1 day up to 14 days post-exposure.

Simulated-use tests

These tests are designed to mimic the practical use situation. For products that knockdown and kill mites simulated-use tests should be performed in which the product is applied according to the instruction for use. When products for general surface treatment are tested the mites must have a choice to be in contact with the biocide or not. The results should be compared to a control test, without biocide.

5.6.4.8.2.3 Requirements per type of claim

Specific mites: when specific mite species are mentioned in the claim (e.g. dust mite, red mite) both laboratory and simulated-use tests are required with the target species.

Mites as secondary pest: When mites are mentioned on the label claim only as a secondary pest, only laboratory tests with one mite species are required.

Acaricides: When mites are the main pest to control both laboratory and simulated-use tests are required with more than one mite species.

Space and structural treatments: requirements for these products are covered in section 5.6.4.11 on stored goods.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.8.3 Assessment of authorisation

5.6.4.8.3.1Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy" (BPD). This is implemented for mites in the following way.

A biocide against mites is normally considered to be sufficiently "effective" if the following results can be achieved:

- laboratory tests: ≥90% mortality in 24 hours;
- simulated-use tests: ≥90%mortality in 1 week;
- field trials for space and structural treatments: requirements for these products are covered in section 5.6.4.11 on stored goods.

Deviations from these norms is possible but should be justified in the application.

5.6.4.9 Fleas

5.6.4.9.1 Introduction

This section covers the assessment of efficacy of products used for treatment against cat and dog fleas. The application of these products is indoors on surfaces.

These biocides are divided into two groups, namely the adulticidal and ovicidal/larvicidal products. Adulticidal products are intended for use against fleas in the adult growth stage, and the ovicidal/larvicidal products for use against fleas in the egg and larval stages. This distinction is based on the very different modes of action of the product, which result in different criteria for assessment.

It should be emphasized that products against fleas, which are applied directly on dogs and cats and have a medical claim are covered by legislation on Veterinary Medical Products. The reader may refer to the borderline dossier available on the ECB website (www.ecb.jrc.it/biocides).

5.6.4.9.1.1 Biology

Of the over 2000 species of fleas (Siphonaptera), the cat flea (*Ctenocephalides felis*) and the dog flea (*C. canis*) are the most common in-home pests in the EU. Fleas undergo complete metamorphosis (egg, larva, pupa, adult) and the lifecycle begins when an adult female finds a suitable host. Once found, the female flea will remain on this host for the rest of its life. Females produce several eggs after each blood feeding and can produce several hundred eggs in its lifetime. Once laid, the eggs fall off the animal host and develop in the areas where the host animal spends its time. The eggs tend to accumulate in the lowest areas such as deep in fibres of carpets, cracks in the floor, or crevices in furniture and furnishings.

Larvae require high protein food for their survival. This protein comes from feeding on the dry faeces of the adult fleas. The adult flea takes in more blood from the host than necessary for nourishment and excretes the remaining blood in almost pure form. Once dried, the faeces falls off the host animal where the larvae can feed. The larvae spin a cocoon and begin the pupal state.

An adult flea emerges from the pupae after stimulation from external cues that indicate an animal host is near. Once emerged, a flea must usually find a host (located using visual and thermal cues) within a week, or it risks death due to desiccation. Complete development from egg to adult occurs in as little as two weeks, but this can take much longer depending on environmental conditions.

5.6.4.9.2 Dossier requirements

A clear label claim should be submitted. The study results of laboratory. simulated-use tests and field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Laboratory and field trials with fleas are needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If there are no guidelines available or the guidelines are not suitable, the applicant may use their own methods, on condition however, that the study is scientifically robust, well

reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.9.2.1 Test species

A product against fleas should normally be tested on the cat flea (*Ctenocephalides felis*) or the dog flea (*C. canis*).

5.6.4.9.2.2 For claims made for products intended for use as general surface treatments

For the evaluation of biocides against fleas different types of laboratory, simulated-use tests and field trials can be used.

Examples of the types of data that may be available when considering the efficacy of insecticide products intended for use as surface treatments are given below.

Laboratory studies

The product is applied to representative surfaces (e.g. carpet discs). Information on the fibre length and density should be provided, as this has a bearing onto flea survival. Long fibres enable fleas to hide and, thus, protect fleas from getting their share of the insecticide applied, Fleas are transferred to the surface, either before (direct contact) or after (residual performance) application of the product, to assess inherent contact toxicity or knockdown effect of the active substance.

Alternatively, ovicidal or larvicidal products can be tested in flea rearing medium containing flea eggs or larvae and the active substance in a range of concentrations, including the intended use concentration. Preferably, tests should be done in five replicates per treatment.

A control treatment without biocide with the same number of replicates should be included in all laboratory trials.

Simulated-use tests

These tests are designed to mimic the practical use situation. The test should be performed according to the label claim.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.9.2.3 For claims made for products intended to be used as space spray treatments

Some insecticides against fleas can be used in foggers. For the evaluation of these insecticides different types of laboratory, simulated-use tests and field trials can be used.

The efficacy test design should be defined for the available treatment method.

5.6.4.9.3 Assessment of authorisation

5.6.4.9.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". This is normally implemented for fleas in the following way.

For laboratory and simulated use:

An adulticidal product against fleas is considered to be sufficiently "effective" if:

- within 24 hours 100% knockdown of the adult fleas should occur (this norm only applies if the test fleas are sprayed directly or are placed immediately on a treated carpet) and;
- within 48 hours ≥90% mortality of adult fleas should occur.

An ovicidal/larvicidal product against fleas is considered to be sufficiently "effective" if:

- ≥80% inhibition should occur of the development of produced eggs/larvae into adult

fleas during the claimed ovicidal/larvicidal duration of action of the product.

Deviations from these norms is possible but should be justified in the application.

5.6.4.10 Litter Beetles

5.6.4.10.1 Introduction

There are several species of "litter beetles" that inhabit poultry droppings and litter. Litter beetles belong to the order Coleoptera, family Tenebrionidae. The most important are the lesser mealworm (other names: darkling beetle), *Alphitobius diaperinus*, and two species in the dermestid genus *Dermestes*; the hide beetle (*D. maculatus*) and the larder beetle (*D. lardarius*). Other species of beetles that occasionally cause damage to poultry housing are *Dermestes ater, Tenebrio mollitor*, *Alphitobius laevigatus*, and *Trox* spp.

Litter beetles are of particular importance as a vector and competent reservoir of several poultry pathogens and parasites. The transmission of bacteria, (*Salmonella, Escherichia coli*) and protozoa (several Eimeria species which can cause coccidiosis) and different viruses can cause problems in livestock. This pest can also cause damage to poultry housing and is suspected to be a health risk to humans in close contact with larvae and adults. Adults can become a nuisance when they move en masse toward artificial lights generated by residences near fields where beetle-infested manure has been spread.

Often these beetles are only mentioned on a label as a secondary pest, while other insects are the main pests (control of flies, cockroaches, and litter beetles in poultry houses). But when they are mentioned specifically on the label they should be tested.

5.6.4.10.1.1 Biology

Lesser mealworm adults lay their eggs in cracks and crevices in the poultry house, in manure or litter, and in grain hulls. Larvae hatch and complete development to the adult stage in 40-100 days depending on temperature and food quality. The larvae consume spilled feed, manure and, to a lesser extent, dead birds and cracked eggs. Beetle populations in broiler and turkey houses often are concentrated around lines of feeders, which provide the beetles with shelter and an opportunity to feed on spilled bird feed. Mature larvae disperse when they are crowded to find isolated pupation sites, and this behaviour is responsible for much of their destructive activity. Crowded larvae leave the litter and tunnel into thermal insulation materials where they construct pupal cells. Both larval and adult stages are omnivorous. The lesser mealworm is nocturnal, with greatest activity of both larvae and adults occurring shortly after dark. Populations of lesser mealworm often reach high densities, especially in deep-litter broiler and turkey houses and in high-rise caged layer operations. It is not unusual for the litter of a broiler house to move from beetle activity or for 70% of the surface of manure in a high-rise house to be covered with adult beetles.

5.6.4.10.2 Dossier requirements

A clear label claim should be submitted. The study results of laboratory, simulated-use tests and field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Laboratory and simulated field trials with litter beetles are needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If there are no guidelines available or the guidelines are not suitable, the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.10.2.1 Test species

A product against litter beetles should normally be tested on the lesser mealworm, *A. diapernus*.

5.6.4.10.2.2 For claims made for products intended for use as general surface treatments

Examples of the types of data that may be available when considering the efficacy of insecticide products intended for use as surface treatments are given below.

Laboratory studies

The product is applied to representative surfaces, either before (persistence test) or after (direct contact) the insects are transferred to the surface, to assess inherent contact toxicity or knockdown effect of the active substance.

Preferably, test should be done in five replicates per treatment.

A control treatment without biocide should be included in all laboratory trials.

Simulated-use tests

These tests are designed to mimic the practical use situation. The test should be performed according to the label claim.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.10.3 Assessment of authorisation

5.6.4.10.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". This is normally implemented for litter beetles in the following way.

A product against litter beetles is considered to be sufficiently "effective" if:

For laboratory and simulated use:

- adulticide: ≥ 95% mortality;
- larvicide: ≥ 95% mortality;
- insect growth regulator: ≥ 90% mortality.

Deviations from these norms is possible but should be justified in the application.

5.6.4.11 Textile-attacking Insects (including fur and fabric attaching insects)

5.6.4.11.1 Introduction

Insecticides against textile-attacking insects can be used by professionals and non-professionals, use against beetle or moth larvae infested carpets for example.

Home user products may be used in vapour phase to prevent moth contact with stored clothing (via killing moth in traps) or insecticides may be applied to the surface of clothing to kill landing moths on contact.

Insecticides against textile-attacking insects can also be incorporated in the textile by industry for preventive treatments.

Other products made from textiles treated with insecticides are the so-called treated articles with an external claim (e.g. carpet with an insecticide not to protect the carpet but against fleas that are in contact with the carpet). These treated articles will not be considered specifically in this section since other than textile-attacking insects are the target insects.

5.6.4.11.1.1 Biology

The two main orders containing textile attacking insect species are Lepidoptera (moths) and Coleoptera (beetles). The webbing clothes moth (*Tineola bisselliella*), fur moth (*Tinea pellionella*), brown house moth (*Hofmannophila pseudospretella*) and carpet beetles (*Anthrenus* sp., *Anthrenocerus* sp.) are common in-house pests that feed on clothing, drapery, carpet and other natural hair fibres. The larvae of these insects have a diet consisting of natural hair fibres, which provide protein from keratin in the hair. These insects have adapted to be able to digest keratin, which is not easily digested by other insects.

Clothes moths are distributed worldwide. They feed during the larval cycle within a silken cocoon attached to hair fibre. Clothes moths larvae that feed only on natural hair fibres such as wool, will not feed on, silk, cotton, linens or synthetic fibres. Adult clothes moths do not feed. These adults mate and the females lays eggs directly on the natural fibre food source.

Carpet beetle larvae (e.g. *Anthrenus* sp., *Anthrenocerus* sp.) attack woollens, rugs and upholstered furniture, etc. The adult beetles, which feed on nectar and pollen, can usually enter the home on plants, flowers or other vegetation. Eggs are then laid on lint in protected areas such as behind baseboards. Once hatched, larvae begin feeding on a number of natural textiles or displays (animal horns, hoofs, insect collections, etc).

5.6.4.11.2 Dossier requirements

A clear label claim should be submitted. The study results of simulated-use tests or field trials should demonstrate the efficacy of the product, based on the submitted label claim.

For vapour based products the label should provide information on the volume that can be covered with the product (closet of x m^3, room of y m^3).

Laboratory and simulated-use trials with textile-attacking insects are normally needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If there are no guidelines available or the guidelines are not suitable, the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.11.2.1 Test species

A product against textile-attacking insects should normally be tested on:

- one of the following moth species:
  - the clothes moth (*Tineola bisselliella*);
  - the fur moth (*Tinea pellionella* L.);
  - the brown house moth (*Hofmannophila pseudospretella*);
- one of the following carpet beetle species:
  - *Anthrenus* sp;
  - *Anthrenocerus* sp.

Whether adults or larvae or both should be tested depends on the label claim.

5.6.4.11.2.2 Laboratory tests and field trials

For the evaluation of biocides against textile attacking insects different types of laboratory and simulated-use tests can be used. Examples of tests, mainly for cloths moth, are listed below.

Laboratory tests

Mortality test
Webbing clothes moths, adults, larvae (2^{nd}-3^{rd} instar) or eggs may be placed in a jar (e.g. 240 ml glass jars, brass-screened lid) containing a treated textile sample (e.g. circular, 4cm diameter, 100% wool sample).

Jars are periodically evaluated by recording mortality, egg laying and hatch (optional), and larval damage. A moth is considered inactivated when it is not able to walk or fly, in a spontaneous way or when stimulated with a brush or pin.

New moths are introduced into the jars periodically to test residual effects (depending on the label claims). Tests should normally be done in five replicates. A control treatment without biocide with the same number of replicates should be included in all laboratory trials.

Simulated use

These tests are designed to mimic the practical use situation. The study results should provide a clear picture of the efficacy of the product.

An example of tests that might match the proposed intended use of the product:

Simulated-use tests with moths added to drawers (minimum air volume: 0.016 m^3) or closets (minimum air volume: 0.5 m^3) can provide good information on home user products. In tests with vapour based products the door should be opened with a frequency resembling normal opening of a closet, to show that this does not reduce efficacy: once a day during completion of the assay, 5 seconds for drawers and 10 seconds for closets. Assessments of mortality would form the basis for efficacy claims. Additionally damage to the test material can be assessed. The damage will depend upon the number of insects, their developmental stage, the exposure time and the size and quality of the piece of carpet, etc. Therefore, damage should always be assessed in comparison to the control treatment.

Simulated-use tests can be waived if a robust field trial is submitted.

Test similar to the ones mentioned above can also be used to show efficacy against carpet beetles and the larvae of carpet beetles.

5.6.4.11.3 Assessment of authorisation

5.6.4.11.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". This is implemented for textile attacking-insects normally in the following way.

At the end of an exposure period (e.g. 1 week) more than 90% of the adults and larvae should be killed (unless claimed different).

Deviations are possible but should be justified in the application.

5.6.4.12 Stored Goods-attacking Insects and Mites

5.6.4.12.1 Introduction

The purpose of biocidal products against stored goods-attacking insects and mites is to control pests in storerooms, freight and alternative transport containers for products of plant origin etc. They should also protect the actual stored goods against insects and mites. The term "stored" in this regard refers specifically to: stored products (of plant origin) for human consumption, animal feed, industrial processing and propagation.

Products against stored goods-attacking insects can either be biocides or plant protection products. In general, where the stored products are protected, prior to processing, the use falls under plant protection and is not relevant in this guideline.

There are a number of different insects that attack stored goods. Common beetle invaders include grain beetles (*Tribolium castaneum, Oryzaephilus surinamensis,* etc.), confused flour beetles (*Tribolium confusum*), and rice weevils (*Sitophilus oryzae*). Indian meal moth (*Plodia interpunctella*) and flour mite (*Acarus siro*) are also very common pest. Infestations of these pests can occur at the packaging plant, the store, or in the home, making it difficult to determine where the source of the problem is. Sometimes these infestations are only noticed by the consumer once the insect leaves the food product and enters the home environment.

For professional and industrial use there are two classifications of such products:

- fumigation with gases, which is used for controlling pests in rooms used for the storage of products of plant origin (storerooms, freight structures and means of transport, gassing installations etc.);
- products other than gases, which are used for controlling pests in empty or full storerooms (including products which are applied by means of vaporisers).

5.6.4.12.2 Dossier requirements

A clear label claim should be submitted. The study results of simulated-use tests and field trials should demonstrate the efficacy of the product, based on the submitted label claim.

Laboratory and field trials with stored goods-attacking insects are needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines

where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. EPPO standards PP 201 to 204 are recommended (Appendix 18). If these guidelines are not suitable, the applicant may use their own methods, on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label.

In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.12.2.1 Test species

A product against stored goods-attacking insects may be tested on beetles, moths or mites (more specifically mentioned in the relevant EPPO guidelines), or insects that are specifically identified in the label claim.

5.6.4.12.2.2 Laboratory tests and field trials

Depending on the application and the purpose of the product, one of the trials below (or equivalent trials) normally should be performed.

Consumer products

For consumer products laboratory or simulated-use tests are required. A direct spray test method can be used to evaluate performance against stored goods-attacking insects. A simulated-use test can be a test, performed in a laboratory, where insects (either cultured or natural populations) are in contact with the stored goods (e.g. breakfast cereal, flour) and the biocide is applied according to the instructions for use.

Simulated-use tests can be waived if a robust field trial is submitted.

A control treatment without biocide with the same number of replicates should be included in all laboratory trials.

Gases for use in storerooms, freight and transport rooms and gassing installations with stored products present

Additional laboratory studies are not required, only field trials.

A field trial should normally be conducted according to the EPPO guideline PP 1/201(1) "Fumigants to control insect and mite pests of stored plant products".

The field of use of the gas are places where large supplies are stored, in particular cereal products, but also other food products such as dried nuts, processed vegetables, spices or meals.

The use of gas can be intended for controlling/fighting pests in spaces but also for controlling/fighting pests in or on the product itself.

Products other than gases for storerooms with or without stored products

Additional laboratory studies are not required, only field trials.

A field trial normally should be performed according to the EPPO guideline PP 1/202 (1) "Space and structural treatments of storerooms".

The products concerned exclude gases, but do include those applied by means of vaporisers (fogs, smokes, vapours, space sprays).

This trial focuses on the control of pests in full or empty storerooms (walls, cracks, etc.). The trial does not serve to test the efficacy of the treatment on pests in the stored products themselves.

The trial can be performed in two ways.

- the first possibility is conducting the trial in rooms where there is already an infestation.

Using a trapping system, the effectiveness is determined by scoring the number of insects caught in the traps before and after the treatment;

- the second possibility is conducting the trial in a room where test organisms have been introduced artificially (usually in small cages). The effectiveness is determined by scoring the number of alive, 'knocked down' and dead organisms in comparison with an untreated room.

5.6.4.12.3 Assessment of authorisation

5.6.4.12.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". This is implemented for stored goods attacking-insect in the following way.

- consumer products: normally 100% mortality in direct spray tests, in simulated-use tests >90% knockdown and >70% mortality after 24 hours would be sufficient;
- gases: the duration of gassing (as specified in the label claim) should be such that at the end of gassing 100% of the insects/mites are dead or dying. It is possible to distinguish between dead and dying insects, which will not recover anymore, so these should also be counted;
- the duration of gassing should not be longer than necessary;
- all non-gases: the effect should be achieved within the duration of the treatment, as specified in the label claim. Normally >90% would be sufficient.

Deviations from these norms is possible but should be justified in the application.

5.6.4.13 Flies

5.6.4.13.1 Introduction

Flies are common pests in and around the house and in animal rearing facilities. Some of these insect species are merely a nuisance, others provide discomfort from irritating bites, and some potentially carry and transmit diseases.

The possible fields of use of the insecticides include: residential and other types of accommodation, public spaces, hospitals, storerooms, kitchens, waste dumps and stables and manure storage facilities.

5.6.4.13.1.1 Biology

House flies (*Musca domestica*) and other nuisance flies are common non-biting pests in the EU. The house fly lifecycle goes through four stages: egg, larvae (maggots), pupa, and adult. Eggs are laid on organic debris including faeces, decaying vegetation, etc. Once hatched, larvae feed by burrowing into the organic debris and filter decaying organic matter. In the pupal stage the fly is transformed into the adult. During this transformation, no feeding takes place. At the adult stage, house flies feed by regurgitating on food, then lap up the food in liquid form. The life cycle of house flies, from egg to fly, is 1 to 3 weeks, depending on the climate conditions. Males die soon after mating, females live temperature dependent normally one to several weeks in the field.

Flies regularly fly into and out of man-made structures. Outside, flies land on faecal material and other debris. Inside, flies land on human food and contact other substrates regularly touched by humans. Here, potential pathogens can be transferred on the flies' body (legs) or from inside the body (vomiting on potential food in order to feed) which are picked up in faecal or other decaying material. More than 100 germs have been documented as being transferred by house flies. Among them are *Salmonella* sp. and *E. coli* have been documented as being transferred by house flies.

The stable fly (*Stomoxys calcitrans*) is a pest often found in stables alone or together with the housefly. Rather unusual for a member of the family Muscidae is that it sucks blood from mammals. Under favourable conditions the stable flies develop from egg to fly in 3 weeks. The adults live several weeks.

Other biting flies include black fly (Simuliidae) and deer and horse flies (*Chrysops* and Tabanids), are also common pests in the EU. These insects can inflict a painful bite leaving an itchy welt. Some are also known to transmit disease. Apart from these species blow-flies can

be of significance in a number of localities, including food producing facilities (Carrion flies, blue bottle fly, green bottle flies).

5.6.4.13.2 Dossier requirements

A clear label claim should be submitted. The study results of laboratory and simulated-use tests and field trials should demonstrate the efficacy of the product based on the submitted label claim.

Laboratory, simulated-use tests and field trials with the test insects are needed to assess the efficacy of the product, depending on the label claim. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If there are no guidelines available or the guidelines are not suitable, the applicant may use their own methods (intra-company Standard Operating Procedures), on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. A control treatment without biocide with the same number of replicates should be included in all laboratory trials.

In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history, season, etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.13.2.1 Test species

In case of an authorisation against flies the prescribed test insect is the housefly (*M. domestica*). When the product claim includes use in stables and animal housings (except poultry), for a general claim against flies both the housefly and the stable fly (*S. calcitrans*) should be tested. If efficacy against blow-flies is claimed tests have to be done with a blow-fly species (Calliphoridae).

5.6.4.13.2.2 Laboratory testing simulated-use tests and field trials

For evaluation of biocides against flies different types of laboratory, simulated-use tests and field trial can be used. Examples of tests are listed below.

Laboratory tests

Flies can be tested in the laboratory in small jars or Petri dishes. The surface can be treated or granules can be placed, after which insects can be added at different time intervals. Alternatively, the flies can be sprayed directly. The knockdown percentages and mortality are determined.

A control treatment without biocide with the same number of replicates should be included in all laboratory trials.

Simulated-use tests

For assessment of efficacy simulated-use tests should be conducted in a test chamber, for instance the Peet-Grady chamber. This is an airtight room of 1.8 x 1.8 x 1.8 m^3, into which a certain amount of product is introduced. Other chambers of similar or bigger size are acceptable, either airtight or with air exchanges. The chamber should be washed and dried between each replicate to avoid chemical contamination.

Environmental conditions must be specified during the test (temperature, humidity, photoperiod). Temperature would be expected to fall in the range 19-29°C, may be lower for use in stables. A control treatment without biocide with the same number of replicates should be included in all laboratory trials.

Simulated-use tests can be waived if a robust field trial is submitted.

Examples of tests for different products are listed below. For other types of products similar test can be performed.

<u>Space treatment</u>

In the case of an application of a liquid for space treatment, the aerosol test method is performed in the test chamber in the laboratory. A known number (50-100) test insects, including males and females, are exposed to the space treatment. The dose sprayed in the chamber should be comparable to the label directions. The test is performed in quadruplicate. A control treatment without biocide should be included. The knockdown percentages and mortality of flies in both insecticide treatment and negative control are determined.

Surface treatment
Products for surface treatment (including window stickers) act on the insect via contact with or feeding from the treated surface. The product can be applied by spraying, brushing, painting, etc. according to the label. These products are also tested in the test chamber.

In the test chamber the product is applied on a small surface or on the whole chamber, in a dose rate appropriate to the label claim. After the surfaces have been left to dry the test can commence. The insects are released in the test chamber at several time points after application (or at least at the maximum residual time claimed at the label), to show residual efficacy. At a suitable period of exposure (e.g. 24 hours) after each test time point mortality of the test insects is recorded. It is mandatory to report temperature and air humidity in the test room. These should agree as much as possible with practical use conditions.

Products to be vaporized or fogged
Only a French recognized guideline (NF T 72-321) is available for efficacy studies with products against flies that should be vaporized (heating element that heats a tablet or liquid, coils, fan driven devices, etc.) or products that should be applied in a fogging treatment. Recently WHO published a guideline for these types of products against mosquitoes. This guideline might be adapted for fly products. Further, the "Large room test" is generally accepted. Other methods are also acceptable if they are scientifically sound and provide a clear picture of the efficacy of the product.

The "Large room test" test can be performed in a non-ventilated room of 20 to 30 m^3. When a ventilated room is used (mimics in some cases reality better) the air exchange should be measured (e.g. one air chamber renovation per hour). The product is applied according to the intended use, allowing it to evaporate over a specified time period (depending on the label claim e.g. 9 hours).

House flies (*M. domestica*) are exposed to the vapour/fog at different time points, e.g. at 0, 2, 4, 6 and 8 hours. The test insect to be used depends on the requested application. At every time point a known number of test insects (e.g. 50), including males and females, are exposed to the vapour. The test is performed in quadruplicate. A control treatment without biocide should be included.

The knockdown percentages (KD50, KD95, KD100), mortality and, if possible, the concentration of the active substance in the room are determined.

Larvicides
Larvicides are often applied to the floor of stables and to manure to prevent maggots and pupa from developing into the next stage. These products can be tested in naturally or artificially infested manure, in boxes covered with gauze. Adult flies emerging from the manure are counted and the difference between treated and untreated manure is analysed. Where IGRs (insect growth regulators) are used as larvicides, it is possible to additionally assess the deformation of larvae and pupae.

Bait products
For products formulated as baits the product should also be tested to establish the intrinsic palatability of the formulation.

The most important factor involved in laboratory testing is to provide a free choice alternative food source to the test insects. The formulation should demonstrate acceptable toxicity in competition with the alternative food source. A control treatment without biocide of similar size as the test itself (i.e. number of replications) should be included in all laboratory trials.

If conducted on both fresh and aged product it may provide information on the storage stability of the product.

Field trials

For application in cattle houses, pigsties and/or treatment of pig and cattle manure for controlling flies, field trials are normally required.

Tests are done preferably during spring and beginning of summer. At the end of summer and autumn population decline might be due to natural causes instead of the insecticide treatment. Apply the insecticide according to the label instructions.

During field trials in stables, special consideration should be given to the choice of the building material (concrete, wood etc.) of the walls and floors of the stables, as well as to the ventilation (number of total air changes per 24 hours), because the conditions should be representative of a practical situation. This can differ per EU country. It is possible to assess whether extrapolation to other types of accommodation is justified. If for example a general registration for poultry houses is requested, but studies conducted in a house for laying hens have been submitted, a rational should be provided that extrapolation is justified.

The effect on the fly population can be determined by counting the numbers of flies (estimation of population size) before, during and after the treatment, or by the differences between treated and untreated objects in the same area. Various assessment methods are acceptable including visual assessments (fly density on a surface or animals is assigned to a category) or quantified measures such as using sticky fly papers, digital photographs of marked areas on walls, collecting dead flies from a defined floor or aisles area etc.

5.6.4.13.2.3 Requirements per type of claim

Per type of claim the requirements will be listed.

Products intended for use as general surface treatment, space treatment or vaporisers in houses:

- a simulated-use test showing mortality and knockdown and/or residual efficacy according to the claim.

Products intended for use as general surface treatment, space treatment or vaporisers in stables and waste dumps:

- a laboratory test showing mortality and/or knockdown and/or residual efficacy, depending on the claim;
- a field trial according to the directions for use.

Products intended for use as larvicides:

- a laboratory test showing larva mortality;
- a simulated-use test showing decrease in number of emerging flies.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.13.3 Assessment of authorisation

5.6.4.13.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". An insecticide against flies is considered to be sufficiently "effective" if the following results can be achieved:

Products intended for use as general surface treatment, space treatment or vaporisers in houses:

- required results in simulated-use tests:
  - the level of knockdown efficacy should be ≥80%;
  - mortality after 24 hour should be >90%.

Products intended for use as general surface treatment, space treatment or vaporisers in stables and waste dumps:

- required results in laboratory tests:
  - the level of knockdown efficacy should be ≥80%;
  - mortality after 24 hour should be ≥90%;
- required results in field trials:
  - reduction in the amount of flies according to the claim (or compared to the control situation).

Products intended for use as larvicides:

- required results in laboratory tests:
  - >90% larva mortality;
  - showing decrease in number of emerging flies.

Deviations from these norms is possible but should be justified in the application.

5.6.4.14 Mosquitoes

5.6.4.14.1 Introduction

Mosquitoes, including species in the Culex, Aedes, and Anopheles Genera are common pests in parts of the EU. As well as their annoying behaviour and itching bites, mosquitoes are well-known for transmitting diseases such as Malaria (Anopheles spp.), yellow fever, Dengue (Aedes spp.), West Nile (e.g. Culex spp.), blue tongue virus in animals, and various encephalitis. Although none of these diseases are endemic in Europe, occasional outbreaks occur and European travellers might encounter them, either in European tropical overseas regions or in the rest of the world. Biocides against mosquitoes can only claim to kill or repel the mosquitoes, not to prevent the diseases.

5.6.4.14.1.1 Biology

Like all Diptera, mosquitoes also go through four stages of development. The egg, larval and pupal stages take place in still aquatic environments such as floodplains, drainage ditches, natural and artificial water containers. Depending on the species, female mosquitoes will lay eggs directly in these aquatic environments or adjacent to locations in mud which typically have fresh water or tidal flooding events. Depending on the genera, eggs are laid individually or in clumps called rafts.

Once larvae hatch, filter feeding begins near the top of the water. Typically, mosquitoes go through 4 larval instars before beginning the pupal stage. Once completed, mosquito adults emerge from the aquatic and enter the aerial environments. Mating usually begins a few hours to days after emergence. Once mated, the females begin to search for a blood meal. Humans and domestic animals are included as potential blood hosts, with some mosquito species preferring human blood to other animals.

Adult female mosquitoes locate potential blood hosts by detecting attractants such as carbon dioxide and skin emanations. Once located, the mosquito will attempt to bite, taking in a blood meal. This blood meal is partially digested and used for the development of eggs.

5.6.4.14.2 Dossier requirements

A clear label claim should be submitted. The study results of trials should demonstrate the efficacy of the product based on the submitted label claim.

Laboratory, simulated-use tests and field trials with the test insects are needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. Several WHO tests are available for mosquito testing. If the available guidelines are not suitable, the applicant may use their own methods (intra-company Standard Operating Procedures), on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. A control treatment without biocide should be included in all laboratory trials.

In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These may include the risk of re-invasion from adjacent areas, general levels of sanitation, treatment history, season, etc. and are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.14.2.1 Test species

In case of an authorisation against mosquitoes insecticide testing should be performed with the house mosquito (*Culex* spp.) since this is the most common in Europe and a large mosquito, which makes it one of the most difficult to kill.

When use in tropical areas is claimed it should be specified against which mosquito spp. the product is effective and these should be tested (e.g. malarial mosquitoes: *Anopheles*).

5.6.4.14.2.2 Laboratory studies

For the evaluation of biocides against mosquitoes different types of laboratory, simulated-use tests and field trial can be used. Examples of test are listed below. Mosquitoes used in all tests should be disease free.

Laboratory tests against adults

Insecticides against mosquitoes should normally be tested in the laboratory in WHO cones or WHO cylinders by force tarsal contact. The test is well described in WHO guidelines (methodology, number, age, nutritional status of the specimens and insecticide susceptibility of the strains). Only females have to be tested. First laboratory test (bio assay) can be conducted on a laboratory strain of well-known insecticide susceptibility. A second test can be conducted on field populations obtained by larval collection. Tests should be conducted on F1 generation adults. Mosquitoes are exposed during a few minutes to a treated surface and their evolution (knock down, death) is followed during 24 hours. The knockdown percentages and mortality are determined.

The cone tests can also be used to evaluate the efficacy of insecticide treated net. For netting evaluation the exposure time is only 3 minutes and mortality is also checked after 24 hours.

Tunnel tests baited with birds or little mammals could be conducted to assess the feeding inhibition and the insecticide effect.

A control treatment without biocide with an adequate number of replicates should be included in all laboratory trials.

Laboratory tests: Larvicides

Larvicides are applied to water to prevent larva to develop into adult mosquitoes. These products can be tested in naturally or artificially infested water, in boxes covered with gauze. Tests are normally not performed in tap water but in water containing organic particles, especially where a claim for residual performance is made. Test is normally performed on late 3rd-early 4th larval stages only. Mortality is usually checked after 24 hours. For slow acting insecticides and insect growth regulators mortality has to be checked for several days. In that case food has to be supplied to larval stages. A control population susceptible to insecticide should be use as control in all bio-assays (positive control). A control treatment without biocide should be included as negative control. Adult mosquitoes emerging from the water are counted and the differences between treated and untreated boxes are analysed. The methodology of this bio-assay is described in WHO guidelines (WHO/CDS/WHOPES/GCDPP/2005.13).

Simulated-use tests

For assessment of efficacy simulated-use tests should be conducted in a test chamber, for instance the Peet-Grady chamber. This is an airtight room of 1.8*1.8*1.8 m, into which a certain amount of product is introduced. Other chambers of similar or bigger size are acceptable.

The chamber should be washed and dried between each replicate to avoid chemical contamination.

Next to chambers experimental huts can be used. These huts are small buildings, several build next to each other, in which wild mosquitoes can enter but they have no way to escape. Volunteers are in the huts as attractants for mosquitoes. In each hut, the treatment of the hut (space or surface treatment) should be different: test product, negative control (no biocide) or positive control (standard product). At the end of a test period (e.g. one night) number of mosquitoes are counted by species, by status (death or alive), by engorgement (fed or unfed) and by position in the hut (hut or exit traps). Advantage of the hut is that wild populations can be used and that it is ventilated (mimics reality better in some cases).

Environmental conditions must be specified at the beginning and during the test (temperature, humidity, photoperiod). Temperature would be expected to fall in the range 19-29°C. When efficacy at high temperatures is claimed (use in the tropics) test at temperatures >30°C should be provided. A control treatment without biocide should be included in all laboratory trials.

Simulated-use tests can be waived if a robust field trial is submitted.

Space treatment simulated-use tests
In the case of an application for a liquid for space treatment, the aerosol test method is performed in the test chamber in the laboratory. A known number (e.g. 50-100) test insects (females) are exposed to the space treatment. The dose sprayed in the chamber should be comparable to the label directions. The test is replicated 3 or more times. The knockdown percentages and mortality of mosquitoes in both insecticide treatment and negative control are determined. Ideally, a ventilated room should be used to mimic the intended use better.

Surface treatment simulated-use tests
Products for surface treatment act on the insect by tarsal contact with the treated surface. The product can be applied by spraying, brushing, painting, etc. according to the label. These products are also tested in a test chamber or an experimental hut. The WHO guideline for testing mosquito adulticides describes such a test.

In the test chamber the product is applied on small surface, or on the whole chamber, in a dose rate appropriate to the label claim. A negative control should be included. After the surfaces have been left to dry the test can commence. The insects are released in the test chamber at several time points after application (or at least at the maximum residual time claimed at the label), to show residual efficacy. After 24 hours mortality of the test insects is recorded. It is mandatory to report temperature and air humidity in the test room. These should agree as much as possible with practical use conditions.

Products to be vaporized or fogged simulated-use tests
No officially recognized guidelines are available for efficacy studies with products that should be vaporized (heating element that heats a tablet of liquid, coils, fan driven dives, etc.) or products that should be applied in a fogging treatment. The "Large room test" is generally accepted. Other methods are also acceptable if they are scientifically sound and provide a clear picture of the efficacy of the product.

The "Large room test" test can be performed in a non-ventilated room of 20 to 60 m^3. When a ventilated room is used (mimics reality better in some cases) the air exchange should be measured (e.g. one air chamber renovation per hour). The product is applied according to the intended use, allowing it to evaporate over a specified time period (depending on the label claim e.g. 9 hours).

Mosquitoes are exposed to the vapour/fog at different time points, e.g. at 0, 2, 4, 6 and 8 hours. At every time point a known number of female test insects (50-100) are exposed to the vapour. The test is replicated 3 or more times. A negative control should be included.

The knockdown percentages (KD50, KD95, KD100), mortality and, if possible, the concentration of the active substance in the room are determined.

When the label claim says that the product should be used in ventilated rooms the opening of windows and doors should be simulated in the test.

Larvicides simulated-use tests

In small scale simulated-use tests, insecticide formulation can be tested in natural breeding sites or simulated larval breeding sites. When natural larval populations are used pre-treatment assessments of the population should done at the site (larval count by dipping technique). Depending on the protocol, eggs or larvae can be regularly introduced in the treated sites to evaluate the residual efficacy. Breeding sites are kept uncovered to allow wild adults to lay their eggs. The methodology of this test is described in WHO guidelines (WHO/CDS/WHOPES/GCDPP/2005.13).

Field trials

For some products against mosquitoes, field trials are not required. Especially when field populations are used in the lab or in an experimental hut. However, for some products and uses a simulated-use test cannot mimic the practical situation sufficiently (e.g. larvicides used

in large swamps and lakes, aerial applications). Especially with aerial applications the way the product is dispersed can make a difference for efficacy. In these cases the competent authorities should require a field trial.

Tests are done preferably during spring and beginning of summer. In autumn population decline might be due to natural causes instead of the insecticide. Larvicides should normally be tested in July-August when sufficient levels of *Culex* spp. and *Aedes* spp. can be found. In any field trial, the assessment of efficacy requires pre- and post-treatment assessments of the population. CDC light traps are one commonly used method to trap mosquitoes and can provide both quantitative (how many mosquitoes) and qualitative (which species are present) data. Other methods (exhauster, aspirator) can be used too. Apply the insecticide according to the label instructions.

5.6.4.14.2.3 Requirements per type of claim

Per type of claim the requirements will be listed.

Products intended for use as general surface treatment, space treatment or vaporisers in houses:

- a laboratory test showing adult mortality;
- a simulated-use test showing mortality and knockdown and/or residual efficacy according to the claim.

Products intended for use as larvicides:

- a laboratory test showing larva mortality;
- a simulated-use test showing decrease in number of emerging mosquitoes;
- depending on the claim (mandatory for use in natural waters) field trial showing larval mortality or decrease in number of emerging mosquitoes.

Simulated-use tests can be waived if a robust field trial is submitted.

5.6.4.14.3 Assessment of authorisation

5.6.4.14.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". An insecticide against mosquitoes is considered to be sufficiently "effective" if the following results can be achieved:

Products intended for use as general surface treatment, space treatment or vaporisers in houses:

- required results in simulated-use tests:
  - the level of knockdown efficacy should be >80%;
  - mortality after 24 hour should be >90%.

Products intended for use as larvicides:

- required results in laboratory tests:
  - 100% mortality after 24 hours of contact is usually required. For slow acting insecticide 100% mortality after 48, 72 hours or more could be considered. Exceptionally a larval mortality >90% can be acceptable if all the surviving larvae died before or during emergence;
- required results in simulated-use test or field trial:
  - >90% larva mortality;
  - showing decrease in number (usually 80%) of emerging mosquitoes.

Deviations from these norms are possible but should be justified in the application.

5.6.4.15 Wasps

5.6.4.15.1 Introduction

There are two types of wasp control: control of the wasps' nest and control of single flying wasps entering a home. The control of wasps' nests may be performed both indoors (in cavity walls or attics), as well as outdoors (in trees, under roof gutters).

5.6.4.15.1.1 Biology

The major pest wasps (Hymenoptera) are the social wasps in the family Vespidae. Yellow-jackets ((*Para)Vespula* spp., *Dolichovespula* spp.), paper wasps (*Polistes* spp.), and hornets (*Vespa* spp.) all belong to this family and are the greatest pests to homeowners. Wasps can be easily differentiated from bees by the fact that a wasp's body appears to be hairless and their hind legs thinner than a bees.

The vespid or social wasp lives in colonies in nests built of a paper-like material. Each nest is begun in the spring by a single queen who has mated the previous autumn. The queen builds a small nest in which she begins to lay eggs. It is only non-fertile female worker wasps that emerge from these initial eggs. These workers take over the nest building duties and forage for food to feed the larvae that emerge from subsequent eggs. Some of these eggs are fertile females and some are males.

Mature colonies are divided into a social order consisting of the queen, workers, males, and fertile females. In the autumn, the males and newly produced queens leave the nest to mate. The male's sole purpose is to inseminate the fertile females, which will become next year's queens. The newly inseminated queens will then find a sheltered place where they will hibernate to begin the cycle with building a new nest the following spring.

Unprovoked, wasps are not aggressive stingers but will protect themselves and their nests making them an undesirable occupant of properties and buildings. Wasps commonly infiltrate in and around homes in search of nest sites and areas to hibernate causing problems for the homeowner. Some people are allergic to wasp venom, and can have life-threatening allergic reactions. Unlike bees, wasps can sting repeatedly.

For effective control of wasps, the entire wasps' nest should be treated. The control is aimed at exterminating all wasps that are within the nest that can fly. If this is achieved, the eggs and larvae that are still present cannot be taken care of and fed anymore, resulting in the elimination of the entire nest.

5.6.4.15.2 Dossier requirements

A clear label claim should be submitted. The study results of field trials should demonstrate the efficacy of the product based on the submitted label claim.

Laboratory and field trials with the test insects are needed to assess the efficacy of the product. Ideally, the studies should be performed according to established guidelines where these are available. These may be international, EU or national guidelines. See Appendix 18 for a list of available guidelines. If there are no guidelines available or the guidelines are not suitable, the applicant may use their own methods (intra-company Standard Operating Procedures), on condition however, that the study is scientifically robust, well reported and provides a clear answer to the question. In addition, the test methods applied and the test conditions should be clearly and fully described and must address the efficacy claim that appears on the product label. A control treatment without biocide should be included in all laboratory trials.

In the case of field trials where true replication is almost certainly impossible to achieve and where normal control methods are not restricted to use of a single insecticidal product, a full description of any factors that might be expected to influence product performance should be given. These are intended to provide the authorities with information to assist with the interpretation of the results obtained.

5.6.4.15.2.1 Test species

A product for use against wasps should be tested on colonies and/or workers of *Vespula* spp. or *Dolichovespula* spp.

5.6.4.15.2.2 Laboratory simulated-use tests and field studies

For the evaluation of biocides against wasps different types of laboratory test and field trial can be used. Examples of test are listed below.

Laboratory tests

Wasps can be tested in the laboratory in small jars or Petri dishes. The individual wasps should have sufficient access to food (e.g. sugar solution), since they can starve to death within hours

when isolated from their nest without food. The surface can be treated, after which insects can be added at different time intervals. Alternatively, the wasps can be sprayed directly. Concentrations used must be in accordance with the claim. The knockdown percentages and/or mortality and/or residual effect are determined.

A control treatment without biocide with a similar number of replications should be included in all laboratory trials.

Field trials

Insecticides with a claim to kill wasps' nests should be tested in a field trial. The efficacy of the product should be tested in at least 5 nests. Depending on the label claim different nests (locations) should be tested (e.g. free hanging in trees or on buildings, hidden in the soil or in wall cavities, etc.). A few like size nests should be monitored over the same test period as untreated controls. A pre-treatment activity count should be taken over a pre-determined time interval of both treated and untreated nests. A well-established parameter for wasp colony activity is the traffic rate, which is defined as the number of wasps entering and leaving the colony in a given time. The traffic rate can be determined 7 days before the treatment for at least 5 minutes at two different times of day as well as on the day of treatment in order get a picture of the colony activity and development. The time interval between both observations must be at least 2 h. Treatment should be consistent with label instructions. When the nest is visible it can be treated directly. In some cases the nest is hidden, for instance in between walls or ceiling of houses. In those cases normally all the openings through which the wasps enter the space in which the nest is hidden should be treated. Nest position, number of entrances as well as wasp species must be described.

After 24 hours, one week and two weeks post-treatment the activity or lack thereof should be recorded by determination of the traffic rate at the treated and untreated nests. The check after one and two weeks is required since it is possible that, when pupae are not eliminated, wasps emerging from pupae can take over the duties of feeding the larvae.

5.6.4.15.2.3 Requirements per type of claim

Products intended for the control of the wasps' nest:

- field trial with at least 5 treated nests.

Products intended for the control of flying wasps:

- laboratory or simulated-use test.

5.6.4.15.3 Assessment of authorisation

5.6.4.15.3.1 Norms and criteria

A biocidal product may only be authorised if it "possesses a sufficient level of efficacy". For wasps this is implemented in the following way.

Products intended for the control of the wasps' nest:

- required results in a field trial:
  - in 80% of the treated nests mortality of the flying wasps should be 100% within 24 hours and all of the treated nests must have 100% mortality (i.e. no visible signs of nest activity) after one and two weeks.

Products intended for the control of flying wasps:

- required results in a laboratory or simulated-use test:
  - ≥ 90% knockdown within a 5 -10 minutes after contact with the product (or according to the claim), direct after spray and at the end of the residual period;
  - mortality according to the label claim, preferably 90% in 1 hour.

5.6.5 PT19 Repellents and attractants

5.6.5.1 Introduction

This chapter covers repellents and attractants under the BPR which are not regulated otherwise, e.g. plant protection products, medical devices, medicinal human and veterinary products. Depending on its field of use a product to repel or attract organisms, e.g. arthropods,

molluscs, leeches, snakes, mammals, birds, etc. may be classified as a biocidal product. Monitoring traps are not in the scope of this guidance.

Efficacy evaluation for attractants in PT18 bait products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

The requirements for efficacy evaluation for PT19 products (except for products against stored goods-attacking insects and mites) do not distinguish between products for professional and non-professional use.

This first section gives a general introduction and lists requirements that are common to all sections. The following sections describe the specific requirements for efficacy testing for each target organism.

Information is missing on some arthropods, molluscs, leeches, snakes, mammals, birds, etc. to be repelled or attracted and also on some of the uses and types of products. In such cases, applicants should design a test protocol and contact their prospective CA to agree upon it. These data gaps will be filled in a future update of this guidance. Nevertheless, efficacy should be demonstrated according to general principles described in this guidance, notably simulated-use test or field trials should be performed.

5.6.5.1.1 Aim

The aim of this document is to provide guidance on how to assess the efficacy of repellents and attractants, in order to ensure that only sufficiently effective products are authorised and therefore placed on the market. Animal welfare considerations are also taken into account.

5.6.5.1.2 Global structure of the assessment

A full assessment of efficacy is required for applications for product authorisations. For active substance approval test protocols described in the relevant sections can be used.

Factors to be considered during efficacy assessment for PT19 biocidal products are the following (non-exhaustive list):

- the target organism (including developmental stage and sex, if relevant) to be repelled or attracted;
- the formulation of the product (e.g. liquid/powder/bait/gas);
- the application method and rate;
- the frequency of treatment and any specific interval between applications;
- other specific conditions (use of the product in conjunction with other activities: e.g. cleaning of an area prior to treatment; contributions made by other components of an Integrated Pest Management plan);
- the areas of use, e.g.:
  - repellents intended for use as topical repellents for human or animal skin;
  - repellents applied on clothing and on other treated articles;
  - repellents applied on surfaces (indoor and/or outdoor environments), spatial repellents (indoor and/or outdoor environments);
  - attractants in traps without PT18 active substances and dispensers (mating disruption).

Information on the effectiveness and intended uses of the product must be sufficient to permit an evaluation of the product and to define its conditions of use.

Efficacy tests (see relevant sections for required test details) should be performed with the product such as marketed for which the authorisation is sought. The studies should demonstrate that the product in the final product configuration (co-formulant(s), packaging if relevant and active substance(s)) is effective for the intended use(s) at the specified dose(s) and use conditions (please note special requirements for Biocidal Product Families, see section 5.6.5.1.3.2). The name and CAS/EC number(s) of the active substance(s) and its percentage(s) used in the test must be provided in the efficacy reports. The assessment will

consider the target organism(s), indoor or outdoor use, the method(s) of application, application rates and use patterns of the product, maximum storage period of the product, together with any other specific claims made for the product. The use(s) specified in the application, i.e. in the draft Summary of Product Characteristics (SPC), submitted by the applicant represent(s) the basis for evaluation.

5.6.5.1.3 Dossier requirements

The following guidance is designed to be flexible and does not specify rigid protocols to which tests must be conducted. Published or unpublished data relevant to the application will be considered if valid. In all cases, the methods and results have to be described in sufficient detail to make the data reproducible and to allow a full assessment. Anecdotal evidence will not be acceptable.

The studies should normally be performed according to established guidelines where these are available. These may be international, EU or national guidelines (ISO, CEN, OECD, WHO, etc.). See Appendix 18 for a list of available standard test methods.

If no guidelines are available or the guidelines are not suitable, the applicant may use elements of their own methods (intra-company Standard Operating Procedures, test protocols or study plans), on the condition however, that the study plan and report are scientifically robust, well reported and provide clear scientifically based results. The test methods and the test conditions must clearly and fully be described and must address the efficacy claim appearing in the SPC.

The following information on effectiveness is required for each biocidal product in accordance with Annex III of the BPR:

- function and mode of action (including time delay, residual efficacy, shelf-life);
- representative target organism(s) (appropriate to the geographic regions the product will be used);
- organisms or objects to be protected;
- effects on relevant target organism(s);
- documentations of undesirable or unintended side effects, for example on beneficial and other non-target organism(s) in case of field trials;
- names of active substances and their respective concentration in the tested formulation and, if needed, e.g. aged product testing, batch number and date of manufacture of the product;
- statement about what is expected from the test, what should be determined and with which precision. Power and sample size considerations should also be included;
- description of test conditions (e.g. size of cage, floor area, presence of harbourages, presence of (alternative) food, water, temperature, photoperiod, location, weather conditions, season, etc.);
- information about acclimatisation of the test organisms (occurrence, conditions, time period);
- number of test organisms (sample size);
- size of the test population in the field before and after the test;
- description of the population composition (e.g. sex, gravid or non-gravid females, nymphs, larvae, age of the population or generation, etc.) if possible, in case of field trials; noting that the feeding behaviour of some insects (e.g. cockroaches) changes during their life cycle;
- information about starvation of the test organisms prior to the test if possible or appropriate;
- description of the history and origin of the test strain, information whether field strains are tested;
- information about all products present in the test, e.g. if, additional to the test product an authorised reference product is present;

- raw data should be available for each study, rather than just a summary of the results;
- show the results of both test (with biocide) and negative control (without biocide) treatment, preferably in a table;
- description of the monitoring methods used before, during and after the test;
- data analyses and statistics including a robust justification for the choice of the used statistical method(s);
- any other information that could be considered important affecting the efficacy.

For all products, efficacy tests have to be done according to the submitted claim and in accordance with any applicable regulations. In the case of tests with vertebrates, it is recommended to contact the prospective CA before conducting the test to avoid unnecessary animal testing due to unsuitable test designs or already existing tests. All experiments using vertebrate animals shall consider the need to avoid distress and unnecessary pain and suffering to experimental animals, in accordance with Directive 2010/63/EU.

A clear claim should be submitted. The study results should demonstrate the efficacy of the product according to the label claim and the use conditions, especially the claimed application rate. In the case of a roll-on, lotion, cream or stick formulation the SPC and the label should provide information on the application rate per surface area, e.g. m^2 or area of the body. Also, for a spray or a product that is applied by fogging the application rate and the number of sprays or the spray duration per surface area or area of the body should be presented on the label and in the SPC.

For vapour-based products, the label should provide information on the volume that can be treated with the product, e.g. closet of x m^3, room of y m^3. Vapour-based products may also serve as surface repellents, and then shall produce protection of a certain area of surface (m^2).

For products applied as surface treatments a claim for residual activity (see 5.6.5.1.4.1.4) is normally valid on the condition that the residues remain undisturbed. This restriction should be included in the instructions for use in the SPC and it should be added that, if applicable the residual efficacy can be lowered by e.g. cleaning, degradation by light and walking. If a residual efficacy after human activities such as cleaning and walking is claimed, then these procedures must be simulated in the context of efficacy testing.

Simulated cleaning should follow the use instructions in the SPC and may include different methods such as vacuuming, wet vacuuming, wet or dry cleaning.

Simulated walking is only relevant for products to be applied to floors in highly frequented areas. Specific uses stated in the SPC e.g. on couches, mattresses or animal beds have to be simulated in the efficacy tests.

In the case of a change, if the change is in any relevant ingredient influencing the efficacy, e.g. adding/removing preservative or change of a food attractant, it should be demonstrated that this modification does not influence the product performance. In all other cases, a scientifically sound justification needs to be provided that the change does not affect the efficacy and the competent authority will then decide if further data is needed.

5.6.5.1.3.1 Biocidal products containing "known active substances" as co-formulants

If the product formulation contains as a co-formulant, so-called "known active substance", e.g. certain essential oils, the reasons for the addition of this co-formulant must be explained. The applicant should submit a scientifically sound justification, possibly based on scientific literature data on the effects of the co-formulant and explain why it is not an active substance in the formulation. If the relevant information cannot be submitted, a study should be submitted showing that the efficacy of a formulation with the active substance but without the co-formulant does not differ from the product containing this active substance and co-formulant.[52]

5.6.5.1.3.2 Biocidal Product Family (BPF)

A BPF may contain several similar repellent/attractant products, which could differ in aspects such as active substance content or colour. If an application is made for multiple products based on the same active substance it may not be required to test all products as long as

[52] CA-Jan18-Doc.4.2_final: Addressing concerns of co-formulants that contribute significantly to a product's efficacy

efficacy is demonstrated for the worst-case product. For example, if two products have identical composition, but differ only in the release rate of the active substance, a test demonstrating efficacy of the product with the lowest release rate will also cover the product with a higher release rate. If specific/different claims are made for these products with regard to the efficacious period, these claims will have to be supported by data.[53]

5.6.5.1.3.3 Treated articles

See section 5.3 for guidance on Treated Articles.

5.6.5.1.3.4 Test design

Although internationally or nationally recognised testing methods are generally preferred, it is not always possible to use these. For some products no standard methods are suitable or available. In that case, a novel test has to be designed.

Various factors must be considered when designing the tests, for example, the number of test individuals (arthropods, mammals, etc.) needs to be chosen. The ultimate aim of relevant considerations should be to design experiments that economise on test individuals, but on the other hand, generate sufficient power to detect effects of a magnitude considered important to demonstrate efficacy. To reduce the overall number of test individuals, replicate tests should be conducted. Another argument for using replicates is to account for the variation among test individuals in susceptibility and responses to the biocides. The numbers of test individuals per replicate group and dose level (treatment group) as well as the number of replicates in the entire study need to be established prior to conducting the tests. As the improvement in power reduces substantially as the number of replicates increases beyond five, it is usually sufficient to conduct five replicate tests at each dose level, employing 10 (or 20) test individuals each. The precise needs will depend on the size of the variances, relative and absolute, between and within the replicates. This can differ between test organisms and test design. Sample size should be adequate to detect differences among groups (negative control vs treated) with a statistical power of at least 80%. Some details on these issues are outlined at the end of each section.

Useful information on the principles of test design, analyses end evaluation of efficacy trials can be found in the EPPO standards, see Appendix 18.

5.6.5.1.3.4.1 Test examples

In the respective sections, examples are given of what kind of tests can be expected for efficacy testing. Sometimes these examples are a summary of a standard test, in other cases, a company test is described or a general idea of what the test should be like is given. There is a great variation in how specific the description is. This is only to give an idea of what kind of tests could be provided. More detailed descriptions of tests can be found in the standard test methods (norms) listed in Appendix 18. This is a list of all available methods (according to current knowledge) without distinction on suitability, usefulness, repeatability, the order of acceptability or robustness. Some norms might have a different approach than described in the section for a particular organism. If this approach is more suitable for the product under investigation the norm should be used.

In all cases, these tests are only meant as examples, not obligatory requirements. Since products are so diverse in application method, mode of action, etc. this guidance cannot possibly cover all testing possibilities for repellents and attractants. Deviations from test designs are possible in adoption to the intended uses and must be explained. Applicants should design a test protocol and afterwards contact their prospective CA to agree upon it.

5.6.5.1.3.4.2 Laboratory trials

Laboratory studies are performed to validate the efficacy in a laboratory test design. Laboratory no-choice tests are not required for product authorisation but can be used for active substance approval to show an innate level of activity for the active substance and to evaluate the right dose. A simple choice test can then be used to see whether a product is effective under ideal conditions. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

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[53] CA-July19-Doc4.2-Final - Note for Guidance: Implementing the concept of the biocidal product family

For product authorisation purposes, a simulated-use test or field trial is normally required, see section 5.6.5.1.3.4.3).

5.6.5.1.3.4.3 Simulated-use tests versus field trials

Simulated-use tests are linked to practical conditions and can, in some cases, be sufficient for demonstrating the efficacy and can include factors like ageing, weathering, UV, washing, etc. Field trials provide a good indication of how the product works in practice/under field conditions, to evaluate how the efficacy can be affected by a variety of factors (the weather, population density, natural fluctuation of the population over time, etc.).

Simulated-use tests or field trials with the test organisms are normally needed to assess the efficacy of the product. Field trials are not mandatory in some cases, as outlined in the sections on specific groups of organisms. In order to eliminate the risk of disease transmission to human volunteers in field settings, field trials with repellents against ticks and mosquitoes are not required for authorisation of products applied on humans or clothing. Pre-existing studies may be submitted as additional information, but field trials are not assessed as key studies for these products. If field trials are conducted, they have to take place in an area with an appropriate density of the target organisms and at a time when the relevant target organisms are abundant. For testing on animals, field trials can be accepted. These trials should be conducted on animals that are exposed to the target organisms anyway so that the studies do not impose an additional risk of disease transmission. Field trials should preferably take place in Europe or other relevant regions according to the claims, e.g. tropical regions. Where testing in Europe is not possible, the conditions and target organisms must be confirmed, and their relevance justified.

Simulated-use tests, depending on the use of the product, can be conducted in indoor as well as in outdoor conditions. In both cases it must be ensured that the tests are conducted under controlled conditions according to the label claim and the use conditions claimed in the SPC (see section 5.6.5.1.4.1.3, further definitions see section 3.1.1 of this guidance).

In the case of field trials, a full description of any factors that might be expected to influence product performance including raw data shall be given. These are intended to provide the authorities with information to assist with the interpretation of the results obtained. These may include general levels of sanitation, treatment history, etc.

5.6.5.1.3.4.4 The importance of negative controls in efficacy studies

The importance of control experiments for efficacy studies must be stressed with regard to the efficacy evaluation. Studies should be conducted alongside negative controls to provide a reference point for the treatment results. A useful definition of this term is given: "A negative control situation may be one in which the experimental design of the study is identical to that of the biocide challenge test except that the biocidal product is not applied or the product without active substance is applied in the control study."

The negative control trial should normally be of similar size, i.e. number of replicates as the test itself, to make statistical comparison possible.

It is recognised that the generation of such control data can be relatively straightforward in well-defined test situations such as laboratory and simulated-use tests. However, it is also recognised that this can present a problem in field situations, where control sites may not be environmentally equivalent to the treatment site.

In such instances, there may be an alternative means of generating reference data other than collecting data from an untreated site. This method may involve pre-treatment monitoring of the site in question. This monitoring must be quantitative, e.g. assessment of numbers of trapped insects. In these instances, a "baseline" infestation level would be established through such monitoring and then the effect of treatment on this baseline can be assessed. Post-treatment monitoring is required for this method.

5.6.5.1.3.5 Use conditions for biocidal products applied on clothing or fabrics

Residual treatments may also involve the claim "unaffected by washing". For the claim "unaffected by washing", the label and the SPC must indicate how often the textile can be washed without reducing the efficacy of the biocidal product. Therefore, clothes or fabrics should be repeatedly washed according to the instructions for use. An ISO 6330 standard for washing performance assessment should be used where relevant, see Appendix 18. Any claims

regarding the efficacy after a certain number of washes should be reflected in efficacy testing after the corresponding number of standardised washing cycles.

The efficacy data should be relevant to prove the submitted claim(s). Therefore, the efficacy of biocidal products must be proven for the whole test individual that should be protected. If only specific body areas intend to be protected, e.g. ears, udders, etc. then the efficacy for the claimed area must be proven.

For specific claims, e.g. prevention of bites through/prevention of bites next to the treated clothes, relevant tests have to be submitted.

Any additional claim referring to wearing conditions, such as "unaffected by UV-light", should be justified by efficacy data. If the claim includes an area other than the area directly treated ("halo effect"), this also needs to be justified by efficacy data.

5.6.5.1.3.6 Mode of action

There are a variety of modes of action and possible effects on target organisms (arthropods, molluscs, leeches, snakes, mammals, birds, etc.) derived from the proposed use of a product to repel or attract. The available data should give brief details to indicate the route and nature of the action, e.g. whether an action is by contact, short or long distance, and the nature of the effect, e.g. sex-specific or unspecific behaviour, attraction, mating disruption. If the mode of action depends on organisms' sex or developmental stage the efficacy trials should be designed to address the most appropriate sex and/or life cycle stage.

5.6.5.1.3.7 Attractants in traps

There are two types of traps which have to be distinguished: traps used to considerably reduce or eliminate the population of the pest species, and traps used for monitoring purposes, i.e. to detect an infestation. Monitoring traps are not in the scope of this guidance.

If the product is applied in a trap, the entire product, including the attractant and trap should be tested. If authorisation is sought for different, but similar, kinds of traps one or more representative traps need to be tested. Variations, e.g. in trap size, shape, colour or material, will be allowed without the need of a special approval when a scientific justification is provided that the change does not impact efficacy. The representative trap of the final product in the application should be as similar as possible to the product to be marketed.

A trap with an added attractant should catch significantly more individuals than one without the attractant.

5.6.5.1.3.8 Proof of non-insecticidal effect for repellent products

It has to be proven that a repellent product at the intended uses does not cause any adverse killing effect to the target organism(s) and its efficacy comes from the repellent, not killing effect. Therefore, the mortality should be determined for repellent products containing active substances approved or under review for both PT18 and 19. For other PT19 active substances, that are only approved or under review for PT19, scientific peer-reviewed literature research should be done by the applicant in order to verify if a lethal effect for the applied target organisms and product usages, i.e. application method and application rate according to the SPC was observed in studies. The methodology and the outcome of the literature research have to be stated in the PAR. If a lethal effect is reported in the literature or no literature research has been done, then tests should be required in order to demonstrate that at the claimed application rate no lethal effect is observed.

The requirements for products against **invertebrates**:

Mortality in the treatment group should be similar to the control group; if mortality in the treatment group will exceed 10%, justification from the applicant is needed.

An additional monitoring step should be included in a simulated-use test, which is described in detail for each target organism in the respective sections. In these tests, the product must be applied according to the instruction of use. Mortality should be compared to a negative control group. Laboratory tests, e.g. filter papers in Petri dishes, are not sufficient to prove the non-insecticidal effect of repellent products, because the test conditions represent no-choice conditions which do not correspond to the application of the product. Field trial can only be used to prove the non-insecticidal effect if the observed population size can be exactly determined before and after the trial. However, any observed mortalities during field trials

should be mentioned in the test summary if it is possible. In addition to the assessment of mortality in the simulated-use test or field trial no further individual studies for testing the mortality are necessary.

The study report should contain information on how mortality was evaluated. A clear distinction between mortality (criteria for the proof of non-insecticidal effect) and moribund/knock-down is necessary for the evaluation. The following definitions can be adapted for the evaluation:

- Mortality refers to dead arthropods that do not move, even when poked or probed after the specific period of time according to the requested test method.
- Moribund or knock-down refers to arthropods that react to stimuli but are unable to move in a coordinated manner, e.g. to upright itself or walk properly. These individuals are observed in the following days to verify if they recover or die.

Products that do not meet these requirements cannot be authorised.

The requirements for products against **vertebrates**:

Mortality due to exposure to the repellent is not acceptable. If some accident occurs during the trial and this is justified in the test report it can be acceptable, but mortality in the treatment group should be similar to the control group and neither group should exceed 10% mortality. No additional efficacy studies for testing the mortality are necessary.

In any case, clinical monitoring, e.g. activity (grooming, movement), weight gain/loss, strength, ataxia, posture, etc. (for more details see OECD guidance ENV/JM/MONO(2000)7 in Appendix 18) of the vertebrate animals is always required, at least for laboratory and simulated-use tests, in order to be sure that the product has no adverse side effects. This has to be documented in the trial report.

Products that do not meet these requirements cannot be authorised.

5.6.5.1.4 Methodology of assessment

Methods of application

When considering the overall evaluation of a proposed claim competent authorities should ensure that the data presented are relevant not only to biological challenge and treatment environment but also that the method of application and application/dose rate(s) used in the test(s) are appropriate to the claims and proposed use of the product.

The application technique should therefore reflect the claims proposed on the label and in the SPC, whether it is a topical application, application on clothing, general surface, crack and crevice or space treatment, spatial repellent, or attractant in traps.

The application method may also describe a specific pattern of treatment. This is particularly common for spray applications but may also apply to other formulation types.

General descriptions of some common treatment patterns are given below.

Surface treatments

These are treatments where the product is applied on surfaces such as walls, floors and ceilings, or used as a treatment on outdoor surfaces. These treatments may involve the treatment of a large area of surface or may only involve an application to a narrow band.

Surface treatments can also include application to temporary or permanent bodies of water, e.g. in mosquito control or to solid and semi-solid manure.

Crack and crevice treatments

These are treatments where products are applied into cracks and crevices where insects hide and harbourage, or through which they may enter the building. Such openings commonly occur at expansion joints, between different elements of construction and between equipment and floors. These openings may lead to voids such as hollow walls, equipment legs and bases, conduits and junction or switch boxes.

Space treatments/spatial repellents

These are treatments where the product is applied to the air rather than onto a surface. They are intended to disperse small droplets or particles into the atmosphere of a room or

other open space, where they will normally stay for a period of time (very small particles may stay in the air for several hours under still conditions).

Spot treatments

These are treatments where products are applied to limited areas on which pests are likely to occur. These areas may occur on floors, walls and bases or undersides of equipment.

General considerations

The efficacy data submitted should demonstrate that the biocidal product, when used as directed by the product label and the SPC, will result in a measurable beneficial effect. The data supplied should demonstrate that an acceptable, consistent level and duration of the intended effect will result from the use of the product at the recommended dose rate.

This may, depending on the individual product, be measured as a reduction, repellency or attraction of the pest population to an acceptable level. The acceptable level may vary depending on the purpose of the proposed use.

Competent authorities should evaluate available data to determine whether they are sufficient to support the proposed claim.

The competent authority will examine the submitted data package and a judgement will be made as to whether any data omissions are considered significant enough to prevent the conclusion that efficacy is proven. Identified data omissions will be communicated back to the applicant. The applicant can then supply additional data or modify their proposed claims in line with whatever has been sufficiently supported.

Any known limitations on efficacy should be considered during the assessment and the following actions taken as necessary:

- State possible restrictions or recommendations concerning the use of the product in specific environmental or other conditions;
- State possible factors that can reduce the efficacy, for instance hot, cold or humid environments or the presence of other substances, in addition to the grounds for these;
- Information on resistance, the likelihood of its development, e.g. scientific literature research, and if needed appropriate resistance management strategies should be provided. Resistance management should be addressed if appropriate guidelines are available, even if it is not to be expected that resistance will build up for repellents and/or attractants;
- State if the product cannot be mixed with, for example, other biocidal products;
- State if the use of the product with other biocidal products is recommended.

5.6.5.1.4.1 Assessment of specific claims

Sometimes a claim will include specific properties of the product, e.g.:

- repels ticks for 5 hours;
- residual effect up to 3 months;
- storage period up to 5 years;
- repels mosquitoes in tropical regions.

Where a particular property is claimed the data submitted to support the product should show that the product actually has these properties. If data do not support this claim, the product may still gain authorisation with amended claims, provided that the product still shows acceptable efficacy.

For example: If a product claims a protection time of 5 hours against ticks, the data submitted must show a repellency for 5 hours after application in order for these claims to be acceptable. However, if the submitted data showed repellency only for 4 hours, the product may still gain authorisation provided that the product claims were amended to “protection time against ticks for 4 hours after application”.

Situations such as the example above will require each study to be evaluated on its own merits, taking into account what the data are actually showing. Evaluators must use scientific judgement to determine when authorisation would not be acceptable.

5.6.5.1.4.1.1 Claims with respect to target organisms

Broad claims, such as "crawling insects" or "flying insects", should normally not be supported, as such claims would cover a diverse range of organisms' habitats, morphology, biology and behaviour. Specific information on the test species for specific organisms are given in the following sections.

In the European tropical overseas regions, the most common genus encountered could be different. A specific claim should therefore be proposed, with referred target organisms. This special request could concern for example special mosquito or cockroach species.

For claims with different target organisms, e.g. cockroaches and ants, efficacy data for each target organism in line with the required species in the specific sections must be provided.

5.6.5.1.4.1.2 Claims relating to storage of a product

For a stated storage period claimed up to two years no tests with aged products are necessary if the physical/chemical analysis ensures that the product composition is still stable after storage and that the active substance content has not decreased more than 10%.

When a product is claimed to be effective after storage of more than two years, it is necessary to demonstrate by analytical studies that the product composition is still stable and that the active substance content has not decreased more than 10%. If the degradation of the active content is > 10% the applicant needs to submit data for effectiveness against all target organisms in a simulated-use test with the product stored at ambient temperature at the end of the maximum storage period. Accelerated ageing studies, in which the product tested is stored under challenging conditions, are not acceptable as these cannot simulate longer storage periods.

For products intended for use as attractant in combination with PT18 (bait products), the efficacy evaluation should be done on the basis of the requirements for bait products[54].

In applications following the simplified authorisation procedure storage stability must either be proven in a storage stability test or in an efficacy test at the end of the stated storage period.

5.6.5.1.4.1.3 Claims relating to outdoor use

When products are intended for outdoor use, e.g. for surface or space treatment or attractants in traps, tests should normally demonstrate efficacy under outdoor conditions. Changes in temperature and rainfall can have an effect on the efficacy of the products. In general, field trials cover this outdoor use. Treated surfaces or products and their negative controls have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified, i.e. temperature, humidity, photoperiod.

For specific uses like "in porches" the conditions in the efficacy test should match with the intended use of the product.

If both "indoor" and "outdoor" uses are claimed for a product, then efficacy studies under outdoor conditions can be used for authorisation of the claim "indoor use", when use instructions (like dose, application rates, frequency) are identical. However, efficacy studies under indoor conditions cannot be used for authorisation of the claim "outdoor use" (exception for mosquitoes and ticks, for details see 5.6.5.1.3.4.3).

<u>Weathering conditions for surface treatment with outdoor usage</u>

Residual efficacy for products with an intended outdoor use should be tested in simulated-use tests or field trials.

When usage in tropical regions is claimed in simulated-use tests then environmental parameters (temperature, humidity, rainfall, solar radiation, etc.) should be adapted to tropical regions. Field trials should preferably take place in tropical regions.

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[54] See Technical Agreements for Biocides - Shelf life of PT18 bait products

Different treated surfaces (according to the claim; e.g. ceramic tile, plywood, stainless steel, concrete) should be exposed to (unless stated otherwise on the label) the following conditions:

- air temperature during storage period must be in the range of 19-29°C;
- rainfall, if necessary, this could be mimicked by artificial watering (examples for a harmonized protocol e.g. from material testing for clothing (EN14360) or wood preservatives (Annex F of EN152), see Appendix 18) for at least 20% of the residual efficacy period (for specific claims e.g. 'rainfast 1hr after application' or 'water resistant' additional testing is considered);
- direct sunlight for at least 30% of the residual efficacy period as most products with outdoor use will typically be applied during the summer months when sunlight is considerable for the majority of the day (6 to 12 hours per day; may depend on the claimed region to be used).

Deviation from these norms is possible but should be justified in the application, e.g. usage under a roof.

5.6.5.1.4.1.4 Claims for residual efficacy

Some pest management programmes involve the use of relatively stable active substances applied to buildings, surfaces or traps to leave residual deposits. These compounds are intended to remain chemically active and therefore effective for periods of weeks and up to several months following treatment, i.e. they have a high residuality. Residual life is a term to describe the period during which the biocide will be present in sufficient quantity to affect a target organism.

Residual efficacy must be proven in tests. Usually, testing is performed to establish the efficacy directly after application and at the end of the residual life of the product.

The types of surfaces to which residual products are applied must be reported since the surface type has a pronounced effect on the amount of active residue available to the target organism. In general, two porous, e.g. unpainted wood, carpet, concrete, linoleum and one non-porous surface, e.g. stainless steel, ceramic tile, vinyl tile, varnished wood should be tested. The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces.

For outdoor use for surface applications, information about exposure time of the treated surface to sunlight has to be provided.

Efficacy data submitted to the competent authority in support for residual treatments should indicate the appropriate dosage and the utility of the formulation when used as directed.

The duration of efficacy, e.g. complete protection time of repellent applied on skin/clothes, residual efficacy for surface treatment demonstrated in efficacy tests should be stated in the SPC and on the label.

5.6.5.1.5 Definitions to determine Complete Protection Time (CPT)

For claiming a CPT, the following definitions are of special importance in understanding this guidance. They apply only in the context of this guidance for products for which a CPT is required (see e.g. 5.6.5.3 Bed bugs, 5.6.5.6 Fleas, 5.6.5.9 Mosquitoes, 5.6.5.13 Ticks) and are not intended to be more generally applicable.

To determine the CPT the target organisms should be exposed to the product every 30 or 60 minutes with the first exposure being 30 minutes after the product application.

Complete Protection Time is the time from the application of a repellent until the last effective observation, before the efficacy failure by a confirmed event. The CPT to be specified corresponds to the time interval before the confirmed event, i.e. the time period in which the product achieves complete repellency against the target organism. For example, at 30 minutes observation intervals, the first confirmed event occurs 4 hours after product application, confirmed by the second event after 4.5 hours after product application. Then the CPT of the product can be claimed for 3.5 hours. At 60 minutes observation intervals, the first confirmed event occurs 4 hours after product application, confirmed by the second event after 5 hours after product application. Then the CPT of the product can be claimed for 3 hours.

A confirmed event is one target organism that is not repelled followed by another similar event within the same or the next exposure period, i.e. 30 or 60 minutes. The first event is confirmed by the second; the second event is the confirming event. In the case where one non-repelled target organism is not followed by another one in the same or the consecutive test interval, then efficacy testing will continue.

An unconfirmed event is one target organism that is not repelled and not followed by another similar event within the same or the next exposure period, i.e. 30 or 60 minutes.

For mosquitoes, a landing is the act of a flying mosquito alighting on human skin without probing or biting. Landing is not always associated with probing. Since a repellent may provide efficacy by a reduction in biting activity but not in landing, this event does not indicate a failure of repellent efficacy. Furthermore, some products do not prevent landing, because the active substances are only effective by direct contact.

The following events indicate a failure of repellent efficacy by testing with mosquitoes:

Probing is the act of penetrating human skin by the mouthparts of a mosquito without ingestion of blood.

Biting is the act of penetrating human skin by the mouthparts of a mosquito with ingestion of blood, typically associated with abdominal swelling and colour change.

CPT calculation

The dataset with CPT for each volunteer should be tested for normal distribution. When results are normally distributed, it may be appropriate to report the mean CPT across all treated subjects with its standard error. Any right-censorship of repellency data, e.g. for one volunteer, the product is effective through the entire observation time, will lead to skewed estimation of both the mean and the variance around it. The use of the median CPT with its 95% confidence limits as the summary measure of CPT is recommended, if right-censorship occurs, see Appendix 18: OPPTS 810.3700.

When the data do not fit a normal distribution—more typical of repellency datasets—it may be possible to transform them to fit a distribution for which a parametric method of analysis can be employed. When the data do not fit and cannot be transformed to fit an underlying distribution, non-parametric analyses, such as Kaplan-Meier survival analysis, are suggested.

5.6.5.1.6 Assessment of authorisation

When considering the overall evaluation of proposed claims, competent authorities should ensure that the data and the method of application and application/dose rates used in the tests are appropriate to the claims and proposed use of the product.

5.6.5.1.6.1 Norms and criteria

The test results are compared directly with the norms and criteria for efficacy described per organism in the respective sections. The performance criteria set in this guidance ask for high levels of efficacy. However, products that do not fully meet the criteria can still be valuable in some cases.

When a product does not perform to the criteria it should be justified in the application why this product is still recommended for authorisation. In a field trial the criteria may not be met because of immigration of insects from untreated areas, e.g. flies, mosquitoes. When this is explained well in a justification the product might still be accepted for authorisation, depending on the results of other field and simulated-use trials.

It should be taken care of that no placebos or misleading products are authorised. If the efficacy level is significantly lower than the criteria stated it should be mentioned in the SPC and on the label e.g. use as Integrated Pest Management, other biocidal products used in combination. The justification will be evaluated case by case. The product should not be authorised unless there is a good reason for having a product of lower effectiveness.

5.6.5.1.6.2 Assessment

The assessor/expert assesses on the basis of the claim and the above criteria. If the product was assessed to be sufficiently effective in simulated-use test and/or field trials, it will be authorised as far as efficacy is concerned.

5.6.5.2 Ants

5.6.5.2.1 Introduction

Ants may cause inconvenience both indoors and outdoors.

In Europe the following ant species are common:

Black garden ant	*Lasius niger* or other *Lasius* species
Pavement ant	*Tetramorium caespitum*
Red ant	*Myrmica rubra*
Erratic ant	*Tapinoma erraticum*
Carpenter ants	*Camponotus* spp.

Next to these native ant species, introduced tropical ants can cause problems, mainly indoors.

Of the tropical and invasive ant species, there are four species that are most commonly found causing inconvenience in buildings in Europe:

Pharaoh ant	*Monomorium pharaonis*
Argentine ant	*Linepithema humile*
Ghost ant	*Tapinoma melanocephalum*
Invasive garden ant	*Lasius neglectus*

This is a non-exhaustive list; other species may be tested if the applicant wishes to claim for a specific target organism.

5.6.5.2.1.1 Biology

Ant development involves a complete metamorphosis that includes distinct egg, larval, pupal and adult stages. Most ant species form colonies comprised of complicated social structures that include infertile female workers, one or more specialised fertile winged queen(s) and (at certain stages in nest development) sexually mature winged males. Some species have developed additional specialised workers that are responsible for guarding the nest and attacking intruders, whilst others perform domestic and foraging duties. These workers will actively forage on a wide range of foods including sweet substances, seeds, insects, aphid secretions, meat, blood and other protein sources. A successful foraging ant also has the ability to communicate where to find food to its co-workers, using chemical signals (trail pheromones). The control of tropical and invasive ants can be complicated by their peculiar colony system: These species are polygynous, meaning each colony contains many queens. Hence, a colony can quickly fragment into several colonies, and colonies do not show aggressive behaviour against each other (unicoloniality) due to a lacking nestmate recognition.

Many native ant species use sand or soil to build their nests, but some also use wood, decaying wood or insulation materials. Some carry water into the nest to keep the right humidity. Such nest-building habits may occasionally cause harm to wooden constructions or insulation.

5.6.5.2.2 Dossier requirements

Dossier requirements are stated in the Introduction, see section 5.6.5.1.3.

5.6.5.2.2.1 Test species

For a general claim "against ants", at least two ant species each belonging to different ant subfamilies (Formicinae, Myrmecinae or Dolichoderinae) have to be tested. For a general claim "against tropical ants", at least two tropical ant species of at least two different ant subfamilies (Formicinae, Myrmecinae or Dolichoderinae) have to be tested. For a species- or genus-specific claim, testing against the claimed species or genus is required.

Due to the specificity of certain active substances, e.g. pheromones, for products based on an active substance with a species-specific mode of action, only effects against ant species that have been tested under simulated-use and/or field conditions, depending on the type of claim, should be claimed on the product label and in the SPC.

National and communal laws might have to be regarded during the evaluation for the use of products against some species such as for example pharaoh ants (*M. pharaonis*).

5.6.5.2.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation (exception see 5.6.5.2.2.2.1 Repellent products intended for use as surface treatment) but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.2.2.2.1 Repellent products intended for use as surface treatment

For product authorisation purposes the efficacy of repellent products should be proven in:

- a simulated-use test according to the instruction for use (test design example see 5.6.5.2.2.2.1.2), or
- a field trial according to the instruction for use (test design example see 5.6.5.2.2.2.1.3) and additionally a laboratory trial testing the required different surfaces.

Products applied onto surfaces may act either by evaporation or on the surface itself.

For products applied on surfaces, two porous and one non-porous surface should be tested, e.g. ceramic tile, plywood, painted plywood, stainless steel, unpainted wood, carpet, concrete for a general claim as "surface treatment". The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces. For authorisation of a product to be used on a specific type of surface the efficacy for only this specific surface should be tested.

For residual efficacy, ants are exposed to the product at several time intervals after application (including the end of the claimed period).

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for detailed information regarding the requirements see Introduction, section 5.6.5.1.4.1.3).

Proposed claims regarding the performance of the product should be simulated in the study. For example, for the claim "unaffected by cleaning/vacuuming", the surface should be repeatedly cleaned during the trial (for details see Introduction, section 5.6.5.1.3).

The repellents used for the test should be identical to the product to be marketed.

5.6.5.2.2.2.1.1 Laboratory test

A choice or a no-choice test can be used, see Appendix 18: Krüger A., Knobelspieß S., and Schmolz E.

General set-up: At least 50 worker ants are placed within a Petri dish, which is standing on a beaker surrounded by water, building an artificial "island". Ants can leave the island via one/two bridge(s) ending in two separate beakers, see Figure 12. Other possibilities to escape should be blocked by a slick insect barrier, e.g. fluon.

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.

Environmental conditions must be specified for the test itself and during the storage of the treated surfaces/substrates or repellent product (temperature, humidity, ventilation, and photoperiod).

<u>Choice test</u>

Ants can leave the island via two bridges of the same material. The formulated product (spray, liquid, gel, powder, dust, etc.) is applied in the middle of one bridge, over the entire width as a stripe; the second bridge remains untreated. Typically, the stripe should be 1 cm wide or according to the claim.

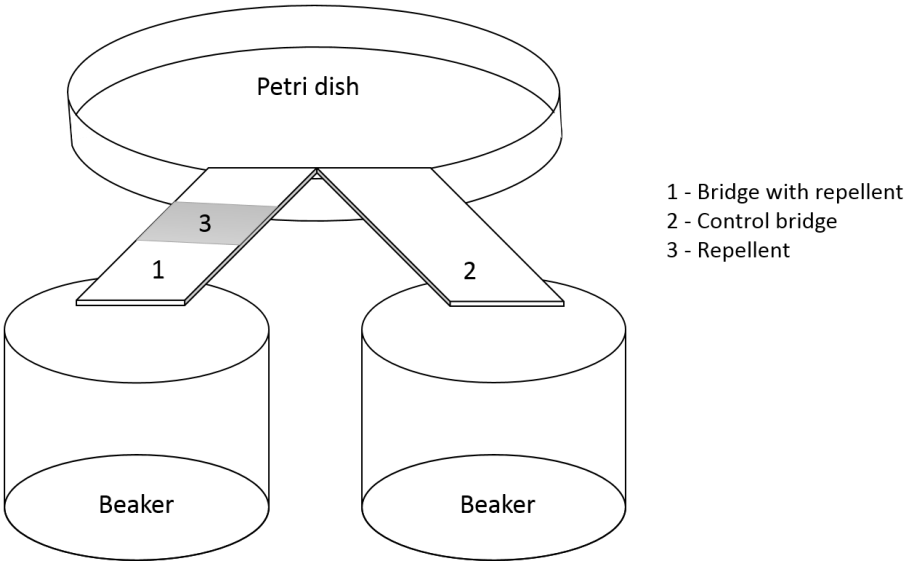

Figure 12: Example for the experimental set-up of the laboratory choice test for repellents against ants

Within the investigation period, e.g. 30 min., the number of ants crossing more than half of the untreated or the treated bridge, by crossing the applied product, is recorded. Subsequently, ants that crossed the middle of the bridge are transferred softly with a paintbrush into the associated beaker at the end of the bridge. To exclude bias due to pheromone trails in the Petri dish the bridges should be swapped among each other after half of the ants crossing the bridges.

To exclude side preference effects, a control treatment without active substance on any bridge should be conducted with insects from the same insect population.

No-choice test

Ants can leave the island only via one bridge. The formulated product (spray, liquid, gel, powder, dust, etc.) is applied in the middle of the bridge, over the entire width as a stripe. Typically, the stripe should be 1 cm wide or according to the claim.

Within the investigation period, e.g. 30 min., the number of ants crossing more than half of the treated bridge, by crossing the applied product, is recorded. In this test set-up some ant species will have to be encouraged to move away from the Petri dish and onto the bridge (by gentle prodding with a soft brush). Repelled ants must be removed from the test system to be able to distinguish clearly the repelled ants from non-running ants.

5.6.5.2.2.2.1.2 Simulated-use test

Mandatory requirements:

- A choice test with complete nests should be performed in a test arena of at least 600 cm^2 total volume (use of two small arenas is possible), see Appendix 18: Krüger A., Knobelspieß S. and Schmolz E.
- Replicates: A minimum of 3 independent replicates (each treatment and negative control) should be performed.
- Each replicate consists of an ant nest, containing workers, brood and queen(s). In case of tropical ants at least 2 queens and 200 workers per colony should be used.
- Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation).
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

Test design: The simulated-use test is designed to mimic the practical use situation. Other test designs than the following example can be accepted if the protocol is scientifically valid.

Ant nests should be introduced into arenas. Arenas should be divided into two similar compartments (Figure 13) by at least a 1 cm wide line of slick insect barrier (e.g. fluon). Alternatively, the ant nest is connected via a bridge to another compartment. Food and water are always placed in the compartments without the nest. During acclimatisation (at least 7 days) ants can reach the food via one bridge and build a pheromone trail. According to the claim the bridges should be of porous and/or non-porous substrate, e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete.

After acclimatisation, the product (spray, liquid, gel, powder, dust, etc.) is applied in the middle of the bridge at the recommended label rate(s) following the product use instructions, covering the entire width in a stripe. Typically, the stripe should be 1 cm wide or according to the claim. Additionally, a second bridge of the same material is provided as negative control. Therefore, ants have a choice of two paths from the nest to food and water, one which they are accustomed to that is now treated, or a new path (Figure 13B). For residual efficacy, ants are exposed to the product at several time intervals after application on the bridges (including the end of the claimed period).

Depending on the size of the ant nest and the ant activity for 1 - 5 minutes (longer observation periods are possible if ant activity is low), the number of ants traveling more than halfway across the treated bridge (over the treated surface) and the control bridge are recorded separately.

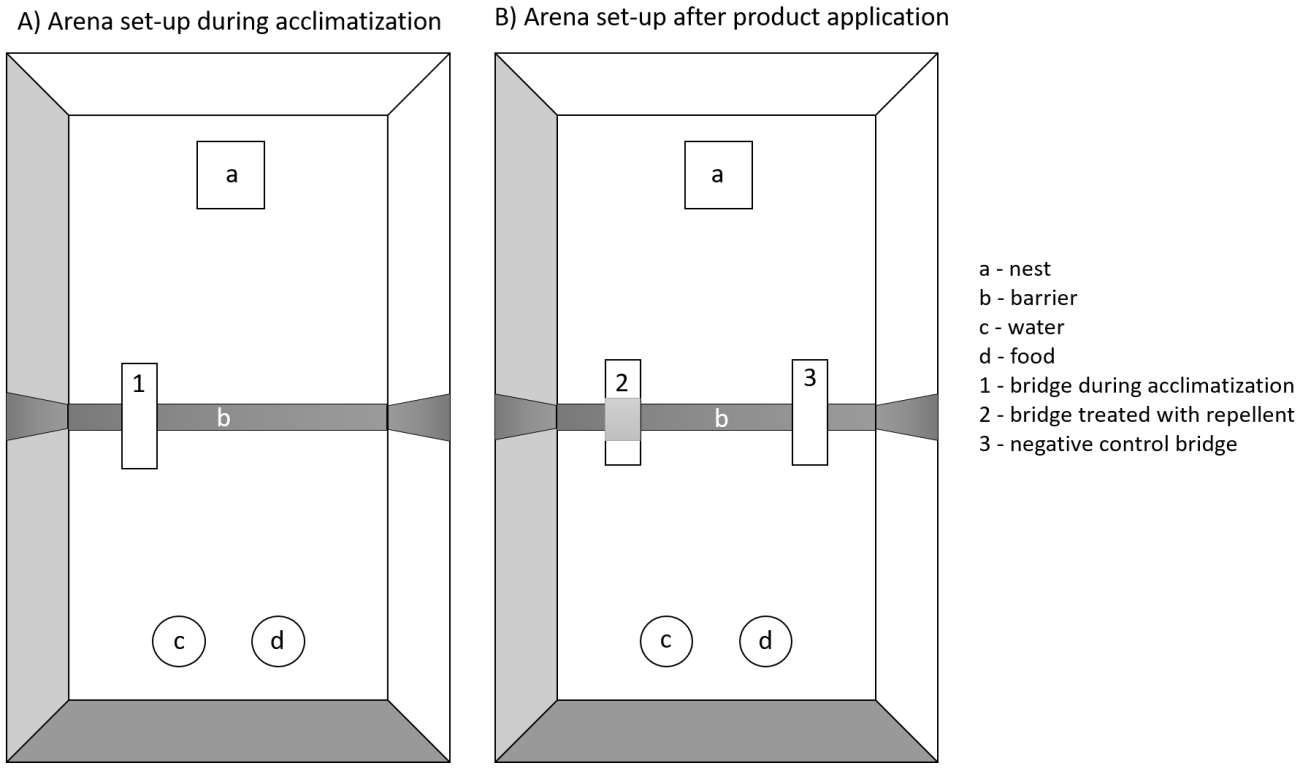

Figure 13: Example for the experimental set-up (A: before and B: after product application) of the simulated-use test for repellents against ants

Proof of non-insecticidal efficacy: After each test run a defined number of ants (approximate 100 workers) should be transferred into a separate arena to monitor the mortality of the workers.

Efficacy assessment: The potential repellent effect of the product is determined by comparing the number of ants crossing the treated bridge vs. the control bridge.

5.6.5.2.2.1.3 Field trials

In the field trials, the product is tested in the actual use situation, for instance in an infested home or garden and applied according to the direction for use in the SPC. Other test designs than the following example can be accepted if the protocol is scientifically valid.

Test design: The efficacy tests against ants should normally be performed on a minimum of three objects with sufficient ant activity (*Lasius niger* 50 ants within 2 min) in each object. The distance between the objects must be large enough to be sure that three different nests will be

observed. For the most ant species three different infested homes or gardens can be used. For field trials with unicolonial ant species (see 5.6.5.2.1) no directly neighboured objects should be used to avoid the testing of only one supercolony. An object can be a place in or near a building, where ants cause inconvenience for the inhabitants. This may be in a house, on a balcony, or on a terrace, depending on the field of use of the product. If the test is performed outdoors, or ants are expected to enter the treated space from outdoors, temperature and rainfall shall be recorded. The product should be applied at the recommended application rate(s) following the product use instructions.

A food source can be introduced near the nest, e.g. 1 meter, for a few days to create an ant trail. To ensure consistent attractiveness, the quality and quantity of the food source must be maintained throughout the trial. A similar neighbour nest must be tested with a food source as a negative control as well. Often ants decide for longer or shorter time periods to neglect food sources, e.g. if aphids occur or the ants focus on protein collection. Before application of the product the time to observe 100 ants must be recorded. This observation period is used to monitor the ant activity after the product application at three different time points.

Monitoring should be conducted at the same locations (as the pre-treatment) and at similar times during the entire trial, e.g. at 12.30, 13.00, etc. Monitoring should continue, e.g. 1 day after treatment, 1 week after treatment, etc. at least once weekly according to the period claimed on the label and in the SPC. A neighbouring nest (similar in size and ant activity) as negative control must be monitored at the same time to document sufficient ant activity.

Replicates: A minimum of 3 independent objects (each treatment and negative control) should be performed.

Environmental conditions must be specified for the test itself, and during the storage of the treated surface/substrate or repellent product (temperature, humidity, photoperiod, ventilation). Timing of trials should be done according to the ant species biology and life cycle. All objects (treatment and control) should be tested during the same period of time and region with a similar ant activity to ensure that tests have not been conducted during the natural decline in ant activity.

Efficacy assessment: The potential repellent effect of the product is determined by comparing the number of ants in the treatment group and the negative control group monitored during the observation period or by comparing the number of ants in the treatment group and the number of ants before the treatment.

5.6.5.2.2.2.2 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractants in traps without PT18 active substance should be proven in:

- a simulated-use test according to the instruction for use (test design example see 5.6.5.2.2.2.2.1), or
- a field trial according to the instruction for use (test design example see 5.6.5.2.2.2.2.2).

Traps should be tested on their own, with a negative control tested separately in an identical test setting.

The attractants, e.g. the trap, used for the test should be as similar as possible to the product to be marketed (for details see General Introduction, chapter 5.6.5.1.3.7).

If outdoor use is claimed, the test should be performed outdoors and the attractant product and control have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

5.6.5.2.2.2.2.1 Simulated-use test

Mandatory requirements:

- A choice test with complete nests should be performed in a test arena of at least 600 cm^2 total volume (use of two small arenas is possible).
- Replicates: A minimum of 3 independent replicates (each treatment and negative control) should be performed.

- Each replicate consists of an ant nest, containing workers, brood and queen(s). In case of tropical ants at least 2 queens and 200 workers per colony should be used.
- Environmental conditions must be specified for the test itself and during storage of the attractant product (temperature, humidity, photoperiod, ventilation).

Test design: The simulated-use test is designed to mimic the practical use situation. Other test designs than the following example can be accepted if the protocol is scientifically valid.

Ant nests should be introduced into arenas. The nest is placed in one half of the arena and food and water are always placed in the other half of the arena. After acclimatisation (at least 7 days) the attractant product/trap is applied according to the claim in the half of the arena containing food and water.

The number of trapped ants is monitored at defined time intervals until the end of the claimed efficacy period, e.g. 24 hours after introduction of the product. For residual efficacy, ants are exposed to the product at several time intervals after application (including the end of the claimed period).

Efficacy assessment: The attraction of a product is determined by comparing the number of ants trapped with the product vs. the control trap to calculate a ratio (product: negative control) of trapped ants.

5.6.5.2.2.2.2 Field trial

In the field trials, the product is tested in the actual use situation, for instance in an infested home or garden, and applied according to the instruction for use in the SPC. Other test designs than the following example can be accepted if the protocol is scientifically valid.

Test design: The efficacy tests against ants should normally be performed on a minimum of three objects with sufficient ant activity (*L. niger* 50 ants within 2 min) in each object. For the most ant species three different infested homes or gardens can be used. For field trials with unicolonial ant species (see 5.6.5.2.1) no directly neighboured objects should be used to avoid the testing of only one supercolony. An object can be a place in or near a building, where ants cause inconvenience for the inhabitants. This may be in a house, on a balcony, or on a terrace, depending on the field of use of the product. If the test is performed outdoors, or ants are expected to enter the treated space from outdoors, temperature and rainfall shall be recorded. The product should be applied at the recommended label rate(s) following the product use instructions.

In the field trial, only those animals that contribute to the infestation in the object are included in the evaluation of effectiveness. Only for a claim "effective against ant nests" the efficacy against the whole ant nest must be proven.

Any bycatch of non-target insects has to be recorded and identified at least to order, preferably to the family level.

Replicates: A minimum of 3 independent objects (each treatment and negative control) should be performed

Environmental conditions must be specified for the test itself and during storage of the attractant product (temperature, humidity, photoperiod, ventilation). Timing of trials should be done according to the ant species biology and life cycle. All objects (treatment and control) should be tested during the same period of time and region to ensure that tests have not been conducted during the natural decline in ant activity.

Efficacy assessment: The attraction of a product is determined by comparing the number of ants trapped with the product vs. the control trap to calculate a ratio (product: negative control) of trapped ants.

5.6.5.2.2.3 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.2.3 Assessment of authorisation

5.6.5.2.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for ants in the following way:

Products intended for use as repellent:

Non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

The required results for the different tests, i.e. laboratory tests (not required for product authorisation), simulated-use tests, field trials are:

- ≥ 90% repellency within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

Attractants without PT18 active substances:

The required results for the different tests, i.e. laboratory tests (not required for product authorisation), simulated-use tests, field trials are:

- at least a ratio of 4:1 of ants trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period;
- ≥ 80% of the estimated population size trapped within the test period (or according to the claim) compared to the negative control), from the beginning and until the end of the claimed efficacy period.

In general for all PT19 products claiming "effective against ant nests" the efficacy against the whole ant nest must be proven.

5.6.5.3 Bed bugs

5.6.5.3.1 Introduction

Bed bugs are small, wingless blood-feeding insects. In temperate climate regions of the EU, *Cimex lectularius* is the dominant species. *Cimex lectularius* and *Cimex hemipterus* are the most relevant species that feed on humans. Bed bugs are not known to transmit diseases in Europe, but even when they are no pathogen vectors, bed bugs can have significant consequences on the health and the quality of people's lives. Bites can cause skin irritation, itching and skin lesions. When the presence of bed bugs is reported, it is necessary to act quickly by calling upon an exterminator to destroy/kill them. A quick and rapid eradication is required due to the high reproduction rate of bed bugs. Only PT18 products are intended to control a bed bug infestation. It is crucial to exterminate bed bugs and prevent the infestation from growing. PT19 products against bed bugs are not intended to eradicate a bed bug infestation. Repellent products against bed bugs can be useful as an accompanying measure in areas where the users cannot exclude a bed bug infestation, e.g. people sleeping in hotels or hostels. In such rooms, the repellent products could be used to protect themselves as well as their luggage.

A sign of bed bug presence includes bites on the exposed skin (small red itchy bumps). Bed bugs usually bite at night while their hosts are asleep. If observed, confined locations such as mattress linings or furniture gaps should be inspected for faecal spotting and the presence of bed bugs.

5.6.5.3.1.1 Biology

Bed bugs belong to the order of Hemiptera, Family Cimicidae. Adults body sizes range between 4.5 mm and 8.5 mm.

Bed bug nymphs, adults and their eggs are found in cracks and crevices in the surroundings of the hosts' sleeping area, e.g. furniture joints, along linings of mattresses, behind paintings and in the seams of furnishings. Bed bugs have a strong aggregation behaviour and leave their hiding place only to feed. They are negatively phototactic, nocturnal and are rarely seen outside the harbourage, except in cases of heavy infestations.

Female bed bugs can lay up to 150 eggs during their lifetime. Depending on the frequency of blood meals, temperature and humidity, bed bugs can have a lifetime expectancy of more than a year. They are able to survive for months without feeding. The nymphs hatch from small white eggs after 7-10 days at room temperature and earlier at higher temperatures. Each of the 5 nymphal stages needs a blood meal to complete development to the next instar. The whole life-cycle from egg to adult takes a minimum of 7 weeks. Blood meals are obligatory for each development stage, egg and sperm production.

5.6.5.3.2 Dossier requirements

Dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

5.6.5.3.2.1 Test species

A product intended for use as a repellent or attractant against bed bugs should be tested against the common bed bug (*C. lectularius*). If the tropical bed bug *C. hemipterus* is claimed, this species should be tested. The origin of the strain has to be documented in the test report, as well as the number of generations for which the bed bugs have been reared in the laboratory (for details see Introduction, section 5.6.5.1.3).

Testing of products that target bed bugs should be conducted with adult and nymph bed bugs. Bed bugs should be tested not less than seven days after the last blood meal.

Due to the specificity of certain active substances, e.g. pheromones, for products based on an active substance with a species-specific mode of action, only effects against bed bug species that have been tested under simulated-use and/or field conditions, depending on the type of claim, should be claimed on the product label and in the SPC.

5.6.5.3.2.2 Requirements per type of claim and test methods

These products will mostly not be able to eliminate an existing infestation and are only useful as a preventive measure. Unless otherwise proven in efficacy trials, the label and the SPC should include a wording like: *"Repellents/attractants should only be used as a preventive measure, e.g. to prevent the spreading of bed bugs via infested luggage"*.

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.3.2.2.1 Repellent products intended for use as surface treatment

For product authorisation purposes the efficacy of repellent products should be proven in a simulated-use test according to the instructions for use (test design example see 5.6.5.3.2.2.1.1).

Test duration should be according to the label claim. If the product is intended to protect the user while in a bed or during sleeping/overnight a minimum efficacy time of 8 hours is necessary to cover the natural bed bug activity over night.

The product should be applied according to the use instructions and the repellent width should be recorded and stated on the label and in the SPC, e.g. "spray a band of at least 10 cm width around the area to be protected".

For products applied on surfaces two porous, e.g. plywood, carpet, fabric, and one non-porous surfaces, e.g. ceramic tile, painted plywood should be used in the simulated-use test, for a general label claim as "surface treatment". The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces. For authorisation of a product to be used on a specific type of surface the efficacy for only this specific surface should be assessed. If a specific surface type is claimed, e.g. textiles, this surface has to be tested.

For residual efficacy, bed bugs are exposed to the product at several time intervals after application (including the end of the claimed period).

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

Proposed claims regarding the performance of the product should be simulated in the study. For example, for the claim "unaffected by cleaning/vacuuming", the surface should be repeatedly cleaned during the trial (for details see Introduction, section 5.6.5.1.3).

The repellents used for the test should be identical to the product to be marketed.

5.6.5.3.2.2.1.1 Simulated-use test for repellents for surface treatment

Mandatory requirements:

- Tests should be conducted with at least 20 bed bugs (10 nymphs and 5 adult females and 5 adult males) per replicate.
- Replicates: A minimum of 5 independent replicates should be performed (each treatment and negative control).
- Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent products (temperature, humidity, photoperiod, ventilation). The room temperature should be kept at 22°C±4°C, with a relative humidity of 30-70%. When efficacy under tropical conditions is claimed, test parameters should be adapted accordingly. If the product is intended to be used in ventilated rooms the simulated-use test should be executed in a room with ventilation.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8), with bed bugs tested within the first hour.

For products which act as contact repellents or at a short distance with a limited release into the air, one of the following set-up examples could be used: an open arena or a three-chambers-system, see Appendix 18, Wang C., et al., or Vander Pan, A., et al. respectively. Both test examples determine if a bed bug is repelled by treated surfaces in the presence of a human host mimic (CO_2 and a heat source). However, for a claim "prevents biting", testing needs to be conducted with human volunteers in order to determine the CPT. Other test designs than the following examples can be accepted if the protocol is scientifically valid.

Simulated-use arena set-up

Test design: In the centre of the test arena (at least 0.6 m^2) a small bed or an imitation with four legs, e.g. a chair or small table is placed. Onto the simulated bed, a CO_2 source (minimum release rate: 100 ml/min) and additionally a heat source (optional) should be placed to mimic a human host. Under each leg, a bed bug interceptor, i.e. a double-walled bed bug trap, where the insects are being trapped between both walls, which form a ring around the bed leg should be placed as a collection vessel. The repellent product should be applied on the outer walls of the four interceptors at the recommended rate(s) following the product use instructions. In case that the test surface is larger than the interceptors are able to represent, then the interceptors can be placed on treated surfaces. Therefore, different surfaces can be treated and placed directly under the legs of the simulated bed. Bed bugs are placed in a harbourage, e.g. a pocket made of paper towel and tape easily opened with scissors in the centre of the arena right under the bed. After acclimatisation (at least 1 hour), the harbourage should be opened. The test arena should be lined with material, e.g. paper and masking tape which enables normal bed bug movements. Inner walls of the arena should be treated with a substance which prevents bed bugs from escaping.

The same test design can be used for the evaluation of products claimed to protect goods e.g. when applied on surfaces like suitcases to prevent bed bug spreading. Instead of a simulated bed, e.g. a suitcase filled with worn clothing or something similar is placed in the centre of the test arena. The repellent product should be applied according to the label claim and SPC, e.g. on the suitcase surface, or as a barrier around the item that is to be protected. An interceptor should be used to collect the bed bugs that cross the border treated with the repellent in the case of a barrier treatment. A harbourage for the bed bugs should be placed in one of the corners of the test arena. After acclimatisation (at least 1 hour), the harbourage should be opened.

Proof of non-insecticidal efficacy: To proof whether insecticidal effects could be caused by contact/exposure to the test repellent product bed bugs tested within the first hour should be used. Mortality of these insects should be monitored 24 hours after the end of the test.

Efficacy assessment: The repellent effect of the product is determined by comparing the results obtained in the treated replicates with the ones from the control replicates. Different methods of recording the efficacy of the products can be used in the test design, e.g. sticky traps, video camera, etc.).

Simulated-use three-chambers-system

Test design: The test system consists of three closed chambers joined with connector tubes. In the first chamber (harbourage chamber), a sealed harbourage with the test organisms is provided, e.g. a pocket made of paper towel and tape easily opened with scissors. After a minimum of 1 h of acclimatisation the harbourage should be opened. In the middle chamber (test chamber) the treated surface is placed. The test chamber is connected to a third chamber (host chamber) containing a CO_2 source and a heat source. The connector tube between the test and host chamber should protrude into the host chamber. A collecting vessel should be placed under the open end of the connecting tube. The collecting vessel should contain filter paper as a harbourage and the inner walls should be treated with a substance that prevents bed bugs from escaping from the vessel.

Efficacy can be evaluated by counting the number of bed bugs which have crossed the surface in the test chamber. The assessment should be based on the counting of the bed bugs that cross the test chamber regardless of whether they are collected in the vessel or still in the tube. If bed bugs are found in the treated chamber or on the treated surface, they should be considered as non-repelled.

To mimic the human host's CO_2 emission, the amount of CO_2 should be adjusted according to the size of the chambers and the distance between each other. Saturation with CO_2 can be avoided by using a suction pump reaching in the harbourage chamber. The heat source inside the test chamber should be adjusted to a temperature of 37°C±2°C. The connector tubes should be lined with material, e.g. masking tape or paper which is not slippery for bed bugs.

Proof of non-insecticidal efficacy: To proof whether insecticidal effects could be caused by contact/exposure to the test repellent product bed bugs tested within in the first hour should be used. Mortality of these insects should be monitored 24 hours after the end of the test.

Efficacy assessment: The repellent effect of the product is determined by comparing the results obtained in the treated replicates with the ones from the control replicates. Different methods of recording the efficacy of the products could be used in the test design, e.g. stick traps, video camera etc.

5.6.5.3.2.2.2 Products intended for use as topical repellents for human skin

For product authorisation purposes the efficacy of repellent products should be proven in:

- a simulated-use test according to the instruction for use (test design example see 5.6.5.3.2.2.2.1), or
- a field trial according to the instruction for use (specifications see 5.6.5.3.2.2.2.2).

The repellents used for the test should be identical to the product to be marketed.

Test duration should be according to the claim. If the product is intended to protect while in a bed or during sleeping/overnight a minimum efficacy time of 8 hours is necessary to cover the natural bed bug activity over night.

5.6.5.3.2.2.2.1 Simulated-use test

Mandatory requirements:

- Volunteers: Efficacy data of at least 10 different volunteers (preferably volunteers with different hairiness of the arms, different genders, age: 18 - 65 years) should be collected since repellence/attractiveness to bed bugs varies considerably between human individuals. For CPT calculation valid data of at least 10 different volunteers must be used.

  Volunteers need to be fully informed about the aim, the procedure, and the expected duration of a study. The study should be carried out in compliance with the national ethics regulation. They also need to be informed about potential side effects such as allergic skin reactions caused by the product. Every side effect observed during the test should be mentioned in the test report. Their participation is voluntary and can be

recalled at any time before and during the study. 12 hours before and during testing, volunteers should avoid nicotine, alcohol, fragrances (perfumes, body lotions, soap, etc.) and repellent products. Prior to the application of the repellent, the skin is washed with fragrance-free soap and rinsed with water.

- Tests should be conducted with at least 4 adult bed bugs per hour per volunteer.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8), with bed bugs tested within the first hour.

A possible test set-up is given below; other test designs than the following example can be accepted if the protocol is scientifically valid.

Test design: The repellent product is applied to human forearms or lower legs. As a negative control, an untreated arm or leg of the same test person will be tested. This also serves to pre-screen bed bugs (adults) for sufficient crawling activity. Bed bugs (adults) showing sufficient crawling activity are used for the test on the treated arm immediately after the control test.

Bed bugs (adults) must be active for host searching behaviour. Therefore, tests should be performed under red dim light during the bed bugs' scotophase (dark phase) and after a starvation period of a minimum of 1 week after the last blood meal. Bed bugs can be put on a piece of cardboard or other material which is held right above the treated skin. Then it is observed whether the bed bugs crawl down onto the skin.

Exposure periods should take place every 30 or 60 minutes with the first being 30 minutes after the product application. 2 to 5 adult bed bugs should be tested on each volunteer during each test period, giving a total of at least 4 bed bugs per hour per volunteer.

A bed bug that does not move at all on the test arm, even after moving on the control arm, has to be excluded from the test, and another bed bug should be used instead. Every bed bug should only be used once for a test to avoid habituation effects. When a bed bug starts to bite during the test, this bed bug should be removed directly from the skin. When a bed bug starts to bite hereby on the treated area this bed bug is considered as non-repelled. Biting can be prevented by constant observation.

Efficacy assessment: While testing for repellence, an endpoint for failure of repellence for subjects treated with the recommended label dose should be selected. Efficacy failure in a test to determine CPT is the time from application of a repellent until efficacy failure by a confirmed event (definition see Introduction, section 5.6.5.1.5). Repellent efficacy should be based on the median or mean CPT.

Proof of non-insecticidal efficacy: Bed bugs tested within the first hour (at least 50 individuals, with 5 individuals per volunteer) are used to proof the non-insecticidal efficacy. The bed bugs should be kept separately in the laboratory and mortality should be recorded 24 hours after exposure.

5.6.5.3.2.2.2.2 Field trials

If the field trials are conducted, they must take place in highly infested buildings with an appropriate bed bug density. At least 30 adult bed bugs should be found within the room upon visual inspection. If no bed bugs are visible, the bed bug infestation should be determined by trapping with a CO_2 and heat source. At least 30 adults should be trapped within 12 hours. These tests should preferably take place in Europe or other relevant regions according to the claims, e.g. tropical regions.

At least 10 volunteers (preferably an equal number of males and females; age: 18 – 65 years) should be included in the field trial, and the same number in the negative control group (see also 5.6.5.3.2.2.2.1 volunteers).

Volunteers must be protected from bed bug bites. This can be achieved by closely monitoring bed bug activity, e.g. under red light, or the use of interceptors.

5.6.5.3.2.2.3 Repellents applied on clothing

For product authorisation purposes the efficacy of repellent products should be proven in:

- a simulated-use test according to the instruction for use (test design example see 5.6.5.3.2.2.3.1), or

- a field trial according to the instruction for use (specifications see 5.6.5.3.2.2.2.2).

Test duration should be according to the claim. If the product is intended to protect while in a bed or during sleeping/overnight a minimum efficacy time of 8 hours is necessary to cover the natural bed bug activity over night.

For residual efficacy, bed bugs are exposed to the product at several time intervals after application (including the end of the claimed period).

Proposed claims regarding the protection of a specific type of fabric must be simulated, i.e. the same type of fabric has to be used in the simulated-use test.

Proposed claims regarding the performance of the product need to be simulated in the study (for details see Introduction, section 5.6.5.1.3.5).

For specific claims, e.g. prevention of bites through/prevention of bites next to the treated clothes, relevant tests have to be submitted.

The repellents used for the test should be identical to the product to be marketed.

5.6.5.3.2.2.3.1 Simulated-use test

For clothing, a test set-up similar to the test described in 5.6.5.3.2.2.2.1 would be suitable; a negative control should be included. Efficacy data of at least 10 different volunteers (preferably volunteers with different genders; age: 18 - 65 years) should be collected since repellence/attractiveness to bed bugs varies considerably between human individuals. For CPT calculation valid data of at least 10 different volunteers must be used. Tests should be conducted with at least 5 adult bed bugs per hour per volunteer.

For other textiles or fabrics a simulated-use test similar to the three-chamber-system could be used (see 5.6.5.3.2.2.1.1). A minimum of 5 independent replicates each with at least 10 bed bugs should be performed (each treatment and negative control). Justify, whether individual bed bugs or groups are tested corresponding to the claim.

Environmental conditions must be specified for the test itself and during the storage of the treated surfaces (temperature, humidity and photoperiod, ventilation). The room temperature should be kept at 22°C±4°C with a relevant humidity of 30-70%. When efficacy under tropical conditions is claimed, test parameters should be adapted accordingly.

Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8), with bed bugs tested within the first hour.

5.6.5.3.2.2.4 Repellent products intended for use as spatial repellents

Spatial repellents can for example be used together with PT18 products, to make bed bugs leave their hiding places and come in contact with the insecticide, see Appendix 18: Benoit, J.B. et al..

For product authorisation purposes the efficacy of spatial repellent products should be proven in a simulated-use test according to the instruction for use.

The repellents used for the test should be identical to the product to be marketed, and the test procedure should reflect the claim.

When the claim states that the product should be used in ventilated rooms the opening of windows and doors should be simulated in the test.

The room size stated in the SPC and on the label must be used in the efficacy trial. For the extrapolation to larger room sizes than proven in the efficacy test a justification is necessary.

For a general claim "spatial repellents" the repellent effect must be proven. For a specific claim "dispelling", the dispelling effect must be proven. If a claim states that the product prevents insects from entering a space, a simulated-use test proving the entry reduction is necessary.

If a claim states that the product prevents insects from biting/probing, a simulated-use test proving the biting/probing inhibition with a volunteer in the room is necessary.

In case of a specific claim regarding the use in specific climate conditions, e.g. high temperature or tropical conditions the efficacy must be proven under these conditions.

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation).

Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

5.6.5.3.2.2.5 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractants in traps should be proven in a simulated-use test according to the instruction for use (see 5.6.5.3.2.2.5.1)

The attractants, e.g. the trap, used for the test should be as similar as possible to the product to be marketed (for details see Introduction, section 5.6.5.1.3.7).

5.6.5.3.2.2.5.1 Simulated-use test

The test should simulate the claimed use of the product. Depending on its use pattern, whether the product is intended to attract bed bugs towards a harbourage or "pull" them away from human hosts, the product performance of an attractant product may be determined e.g. by comparing its effect on bed bugs to that of bed bug aggregation cues or host cues such as carbon dioxide and body heat. Therefore, in the experiment either host cues such as carbon dioxide and body heat or bed bug aggregation cues deposited in a harbourage should be presented as an alternative to the attractant formulation in the same arena. Observations of bed bug location should be recorded at the end of the exposure period. The exposure period should be according to the SPC and label claim. Testing in the dark under red light is recommended.

Replicates: A minimum of 5 independent replicates each with at least 20 bed bugs (10 nymphs, 5 adult females, 5 adult males) should be performed (each treatment and negative control). Justify, if individual bed bugs are tested instead of groups.

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or attractant product (temperature, humidity, photoperiod, ventilation). Room temperature should be kept at 22°C±4°C, with a relative humidity of 30-70%. When efficacy under tropical conditions is claimed, test parameters should be adapted accordingly.

Any bycatch of non-target insects has to be recorded and identified at least to order, preferably to the family level.

5.6.5.3.2.2.6 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.3.3 Assessment of authorisation

5.6.5.3.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for bed bugs in the following way:

Repellents against bed bugs:

Non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

Test duration should be according to the claim. If the product is intended to protect while in a bed or during sleeping/overnight a minimum efficacy time of 8 hours is necessary to cover the natural bed bug activity over night.

For a claim "prevents bites" or "prevents the spreading of bed bugs", e.g. for luggage, 100% repellency are required and the CPT needs to be proven.

For repellent products intended for use as surface or spatial treatment:

The required results for laboratory test, simulated-use tests or field trials are:

- ≥ 80% repellency within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

For products intended for use as topical repellents for human skin and repellents applied on clothing:

The required results for laboratory test, simulated-use tests or field trials are:

- during the claimed protection period complete protection should be proven expressed as CPT (for details see Introduction, section 5.6.5.1.5).

Attractants without PT18 active substances

The required results in the laboratory test or the simulated-use test are:

- at least a ratio of 4:1 of bed bugs trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period;
- ≥ 80% of the test individuals trapped within the test period compared to the negative control (or according to the claim), from the beginning and until the end of the claimed efficacy period

Test duration should be according to the claim. If the product is intended to protect while in a bed or during sleeping/overnight a minimum efficacy time of 8 hours is necessary to cover the natural bed bug activity over night.

5.6.5.4 Biting midges (*Culicoides*, veterinary)

5.6.5.4.1 Introduction

Biting midges (Diptera, Ceratopogonidae, *Culicoides* Latreille) are well known veterinary disease vectors and nuisance in livestock. They transmit a wide range of filaria including *Onchocerca cervicalis* in Europe. A range of viruses has been isolated from biting midges including *African horse sickness virus* (AHSV), a highly infectious disease that may causes serious lung congestion and may lead to the death of horses. This disease is mainly reported from Spain and Portugal. Also, the closely related epizootic haemorrhagic virus and the *Bluetongue virus* (BTV) are transmitted by biting midges. BTV affects ruminants such as deer, cattle, sheep and goat and is transmitted by species like *Culicoides imicola*, *Culicoides obsoletus*, and others. Apart from *Culicoides* biting midges role as vectors they can also represent a serious nuisance. Horses may suffer from equine summer eczema (sweet itch) caused by an allergic reaction to saliva from biting midges. The mane, back and tail areas are often affected, but it can be seen all over the body. Ponies and islandic horses are more sensitive to sweet itch than other horses. Cattle may suffer from the mere numbers of biting midges attacking in evenings with no wind, and high temperature and humidity in areas close to the biting midges breeding areas.

5.6.5.4.1.1 Biology

Culicoides biting midges are the smallest members of the blood-feeding Diptera with sizes of 1-4 mm. They have broiled or spotted wings which characteristically are found directed backward when at rest. *Culicoides* biting midges are recorded from all over the world apart from the most extreme Arctic and Antarctic regions. There are over 1400 *Culicoides* species in the world and at least 83 species are described from Europe. Biting midges feed blood on a relatively wide range of mammals and birds. Only the females are blood-suckers. The activity of the different species varies through the season. Some have a single annual activity optimum, others have two while some are present throughout the season. The periods of abundance have great importance for those species that are vectors for pathogens. The female midges must have blood meals to develop eggs and they need new blood meals for each of their ovarian cycles. This means that a female must seek and find a new host several times in her lifetime. The *Culicoides* biting midges fly mainly in the dusk but sometimes also early in the morning. In rare cases, they attack in the middle of day if the conditions are suitable i.e. low wind, high temperature and high humidity. Various *Culicoides* species are to some extent host-specific in their blood-feeding, some favour mammals, some birds and some amphibians. Many species of *Culicoides* show a different preference for blood-feeding on individual host animals; some favour to feed the belly and udder region on cattle, while other species prefer the extremities, the back, or the head.

5.6.5.4.2 Dossier requirements

Dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

5.6.5.4.2.1 Test species

As of now, only two biting midge species, i.e. *C. sonorensis* and *C. nubeculosus* can be raised in the laboratory, and probably none of them can act biologically relevant in captivity when sensitive biological parameters such as host-seeking is investigated. The important issue, when the efficacy of a repellent is measured, is whether a product, when applied to the host in a relevant environment, can overrule all the key stimuli that attract biting midges to their host. Tests with laboratory colonies of relevant *Culicoides* species as well as with field collected specimens are applicable provided such testing is justified in detail.

- Products intended for use as topical or spatial repellent should be tested with the claimed host, e.g. cattle, horse, sheep.
- Products intended as a general *Culicoides* biting midge repellent; in case of a general claim against biting midges, repellent testing should be performed with biting midges present on the hosts in field trials or simulated-use test. Specimens can be collected during the trial with aspirators on the control animals for later identification in the laboratory. Alternatively, species from the study area are identified previous to the study.
- If a specific species is claimed, then efficacy against this species must be demonstrated.
- Products intended for use as attractants should be tested against claimed *Culicoides* species.
- Test organisms should belong to species encountered in Europe or European territories (overseas territories). In addition, efficacy against other organisms not encountered in Europe or European territories should be demonstrated on the relevant species if claimed. The climate conditions (temperature, humidity) should be representative to European conditions.
- When use in tropical areas is claimed, test at temperatures > 30°C should be provided. Additionally, it should be specified against which *Culicoides* biting midges the product is effective, and these should be tested.
- In case of specific claims, e.g. effective at high temperature, in contact with water, host sweating, etc., efficacy must be demonstrated in the relevant situations.

5.6.5.4.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/ attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.4.2.2.1 Products intended for use as repellents

The efficacy of a product should be shown in either a robust simulated-use test, where animals can be tethered and observed close by or a field trial where the animals are loose/untethered and observed from a distance to establish the efficacy and persistence of the product when used according to the label claim in a real-life situation.

For PT19 products which are applied on animals, no common protocols are available. However, the European Medicines Agency (EMA) has published several guidelines for the evaluation of ectoparasiticides, see Appendix 18. These guidelines may be adapted for the demonstration of the efficacy of repellent products to be applied on animals, but any well documented protocol can be used, provided it is scientifically sound.

It is important to give a detailed description of the circumstances in which the experiments are conducted, with special emphasis on the ratio of treated to untreated host animals at each trial location and trial replicate. It may be of consequence for the trial outcome whether all hosts in a herd are treated, compared to a situation where only a minor part of the hosts in a herd is treated. It is also of importance that the diurnal rhythm of biting midge activity is taken into consideration in the setup of the efficacy trials and reported in detail. If the product is part of a push-pull strategy, e.g. hosts with repellent combined with attracting traps, then such a setup must be reported in detail as well.

If treated horse rugs or similar covers are claimed to be effective in the field, then it must be demonstrated effective in the field. It is not important to make an evaluation of the biting midges on the rug itself, but the emphasis must be on the exposed parts of the host such as the neck, head, legs and belly, including the sheath on males and udder on females, which are thin-skinned areas and very attractive to several blood-feeding species.

Tests must be presented with all host animal species claimed. Bridging efficacy data from one host species to another can be accepted based on robust scientific justification taking into account e.g. the difference in dosing between species (a small goat vs. a large cow), whether sweating is possible or not.

5.6.5.4.2.2.1.1 Laboratory test

Laboratory tests of repellents are not advised, as the differences in food seeking biology of the relevant species are huge. It would be possible to do laboratory tests with a few biting midge species as they can be kept in the laboratory, and therefore specific claims on these species may be backed by data generated in laboratory experiments, but it will not be possible to extrapolate such data to cover any other species encountered on grazing livestock.

5.6.5.4.2.2.1.2 Simulated-use test

In a simulated-use test different parameters are controlled. A simulated-use test of a repellent with full control of host animals and absence of their normal avoidance behaviour can be used to evaluate the efficacy and the persistency of the treatment; topically, spatial or impregnated textiles. The product should be applied according to the claim. Environmental conditions must be specified at the beginning and during the test (temperature, humidity, photoperiod). No specific temperature or humidity are recommended, but the environmental conditions must be suitable to have biting midge activity high enough to challenge the product. A control treatment without repellent should be included in all trials to secure a measure of *Culicoides* biting midge activity.

A simulated-use test must be conducted at a time in the season, as well as on days where the relevant *Culicoides* species are active in the field. Depending on the target animal species, several factors, e.g. hair length, thickness of the coat, grooming, etc. might impact the efficacy of topical repellents. Explain in the test report/documentation the circumstances around the choosing of the test animals. If animals of different colour are chosen be sure not to have a majority of light coloured animals. Ideally, light coloured animals are not included, but state on the label if the product is for a specific colour of animals if only light coloured animals were tested a sound justification is needed to include all fur colours in the claim. For products only tested on e.g. pied (black or red) cattle, it can be necessary to state on the label that the product is for pied (black or red) cattle. For products tested on sheep, it can be necessary to test on both sheared and unsheared sheep, a sound justification is needed if only sheared or unsheared sheep are tested.

At least two groups of host animals must be used, one group treated with the product and one group with no treatment. At least ten different individuals are suggested to be used in each group. In the case of horses, a setup could be 2x5 horses with a treatment switch after e.g. a week, depended on the residual efficacy of the tested product, tested at one location, or 2x5 animals tested at two different locations. At the end of the test period, there must be 2x10 valid datasets. Each treatment group should be housed in separate enclosures throughout the course of the trials to prevent cross infestation or cross-contamination.

Preferably, no test animals should have a recent history of treatment with insecticide/acaricide or antiparasitic agents. Concerning endo-antiparasitic agents this is acceptable, but details (product, dose, application rate, application method, date of the last treatment, and residual efficacy) must then be given in the report; the controls and the treated groups must have the same status. A safety margin for externally applied anthelmintics is the end of last application +1 month. The history of the treated and the control groups should be comparable.

Animals should be treated with the test product once the infestation has become established. The infestation should be documented by e.g. recordings of the numbers of biting midges or photographs. As mentioned above specific *Culicoides* species tend to prefer specific areas on the hosts. Therefore, collections or counts must be conducted in the same area on all hosts i.e. on the back, or on the belly etc. It is acceptable that counts/collections are done on tethered hosts. If the product is acting at very close range it may be possible to make the evaluation by

treating parts of the host, like one treated side of the belly compared to the equivalent area on the other, untreated, side of the belly. The advantage of such a setup is the neutralisation of individual host differences in biting midge-attraction. Collection can be done using an electrically driven aspirator in a defined time period. The attractiveness of the test animals or experimental groups should be tested prior to treatment.

Percentage inhibition (landing) is based on the number of biting midges landing on the untreated control animals and the number of biting midges landing on the treated animals:

Pct inhibition: $((Landing_{Control} - Landing_{Treated})/Landing_{Control}) \times 100$.

Alternatively or in addition to midge landing catches, also the frequency of certain horse's avoidance behaviour according to Mottet et al. (2018), see Appendix 18, can be recorded as a substitute, provided the abundance of the target species predominantly causing the avoidance behaviour is measured at the beginning and the end of the trial. Test designs may also be adapted from Japin, M. and Haanen, G. A. Y.(2013), see Appendix 18.

5.6.5.4.2.2.1.3 Field trials

In a field trial the animals must have the opportunity to display normal avoidance behaviour considering how the product is intended to be used. The tests may be conducted as described for the simulated-use test above, with the adjustment that there is no interference with the host animal's normal behaviour in the field.

The field trials should be conducted on two different geographical locations.

At least two groups of host animals must be used, one group treated with the product and one group with no treatment. At least 10 different individuals are suggested to be used in each group. Due to feasibility and cost-effectiveness, it is possible to use 10 animals in one week (5 each in the treatment and control group) and then use the same individuals again in the second week, but swap treatments. Each animal would then act as its own control. Any residual activity of the product has to be excluded. The field trials can be performed twice with 5 animals (in each of the control and treatment groups) each time at the same location, or with 5 animals (in each of the control and treatment groups) at two different locations. At least 10 animals (each as control and treatment) must be tested. The two locations can be in the same country or region, e.g. two villages. In the case of cattle, the test can be performed at only one location using 10 cows for the control and 10 cows for the treatment.

As documentation for persistency, counts are repeated with chosen intervals of lengths depending on the claim. If there is a claim for use on wet/sweating hosts, then data must be provided showing that the product is effective under such circumstances. The guideline from the EMA: "Guideline on specific efficacy requirements for ectoparasiticides in cattle", and Herholz et al. (2016), see Appendix 18, may be of inspiration for field trial setup.

5.6.5.4.2.2.2 Attractants without PT18 active substances

Use of an attractant in a trap in a push-pull setup may be suggested. A trap could be loaded with CO_2, heat, octenol, butyric acid, etc. In such a setup the biting midge load will be measured as described above.

For product authorisation purposes, the efficacy of an attractant in a trap without an active substance according to PT18 should be proven in a simulated-use test or a field trial according to the instructions for use.

The attractants, e.g. the traps, used for the test should be as similar as possible to the product to be marketed (see Introduction section 5.6.5.1.3.7) and the trap should be designed in a way that reduces bycatch to an absolute minimum. Traps should be tested on their own, with a control tested separately in a similar test setting.

5.6.5.4.2.2.3 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.4.3 Assessment of authorisation

5.6.5.4.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for biting midges on livestock in the following way:

Products intended for use as repellent on animals (including a delivery system)

- A simulated-use test or a field trial demonstrating ≥ 80% repellency within the test period (according to the claim), directly after product application and at the end of the claimed use period;
- All claimed target host animals must be tested. Claims on herds as well as individuals are acceptable and should be tested as such;
- In case of specific claims, e.g. effective at high temperature, in contact with water, when the host are sweating, when horses are at work etc. these should be demonstrated.

Products intended for use as a spatial repellent or for use as attractants without PT18 active substances

A trap with an added attractant should catch significantly more biting midges than one without the attractant. The traps used for the test should be as similar as possible to the product to be marketed.

- A simulated-use test or a field trial demonstrating ≥ 80 % efficacy (i.e. 80% of the test individuals trapped) within the test period according to the claim, directly after product application and at the end of the claimed use period;
- All claimed target species must be tested;
- At least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

The product label and SPC should state that the entire insect population might not be caught/repelled by this type of product; the label claim should be "reduces" and not "protects"/"protection", with no mention of a CPT.

5.6.5.5 Cockroaches

5.6.5.5.1 Introduction

Cockroaches are a common and persistent problem in many households, commercial premises, domestic buildings, private and public areas, restaurants, industry. These crawling insects (although several species with the ability to fly) are scavengers, allowing them to readily adapt to changing food availability. Cockroaches may act as a mechanical vector of various pathogens, e.g. can carry bacteria such as *Salmonella* in areas co-inhabited by humans. Cockroaches are also identified as a major cause of allergies and asthma, particularly in children. Amongst the crawling insects, cockroaches are one of the most difficult to control.

5.6.5.5.1.1 Biology

Cockroaches belong to the (sub-) order Blattodea. There are over 3500 species of cockroaches, but only a few are considered domestic pests in the EU. The German cockroach, *Blattella germanica*, the Oriental cockroach, *Blatta orientalis*, and the American cockroach, *Periplaneta americana*, are the most common.

Upon hatching from an egg capsule, cockroaches begin their nymphal stage and moult through various instars until reaching the adult stage. The nymphal stages are smaller than the adult stage without fully developed wings and sex reproduction organs. The time of development can take weeks or months depending on the species and the surrounding environmental conditions. Depending on the temperature, the eggs of the German cockroach hatch after 3 to 5 weeks, the nymphal development with 5 to 7 moultings takes 40 days to 6 months and the adults live for about 6 months.

In temperate European countries, most cockroach species will almost never be found outside, with foraging activities almost entirely within human-made structures.

5.6.5.5.2 Dossier requirements

Dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

5.6.5.5.2.1 Test species

For a general claim "against cockroaches", two key species should be tested: one small species, belonging to the family Ectobiidae, the German cockroach *B. germanica*, and one large species, belonging to the family Blattidae, either the Oriental cockroach *B. orientalis* or the American cockroach *P. americana*. For a specific claim, testing against the claimed species is required.

Due to the specificity of certain active substances, e.g. pheromones, for products based on an active substance with a species-specific mode of action, only effects against cockroach species that have been tested under simulated-use and/or field conditions, depending on the type of claim, can be claimed on the product label and in the SPC.

For insects from laboratory rearing used in the efficacy studies, age and feeding condition should be reported (for details see Introduction, section 5.6.5.1.3).

5.6.5.5.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.5.2.2.1 Repellent products intended for use as surface treatment

For product authorisation purposes the efficacy of repellent products should be proven in:

- a simulated-use test according to the instruction for use (test design example see 5.6.5.5.2.2.1.1), or
- a field trial according to the instruction for use (test design example see 5.6.5.5.2.2.1.2) and additionally a laboratory trial testing the required different surfaces.

A simulated-use test testing the product and the negative control in one set-up can only be used as additional, supportive information. Testing of the product and the negative control separately is necessary.

Products applied onto surfaces may act either by evaporation or on the surface itself.

For products applied on surfaces, two porous and one non-porous surface should be used, e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete, for a general claim as "surface treatment". The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces. For authorisation of a product to be used on a specific type of surface the efficacy for only this specific surface should be assessed.

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range of 19–29°C. When efficacy at high temperatures is claimed, 40°C should be the test temperature.

For residual efficacy, cockroaches are exposed to the product at several time intervals after application (including the end of the claimed period).

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for detailed information regarding the requirements see Introduction, section 5.6.5.1.4.1.3).

Proposed claims regarding the performance of the product should be simulated in the study. For example, for the claim "unaffected by cleaning/vacuuming", the surface should be repeatedly cleaned during the trial (for details see Introduction, section 5.6.5.1.3).

The repellents used for the test should be identical to those used in the product to be marketed.

5.6.5.5.2.2.1.1 Simulated-use test

Mandatory requirements:

- In the simulated-use test that evaluates the repellent efficacy of products intended for use as surface treatment the insects must have a choice to be in contact with the repellent or not Product and negative control are separately tested.
- Conducted in arenas with a surface of at least 0.5 m^2 (intended as whole surface if the test is performed connecting 2 arenas).
- The test arena should be equipped with water, food and a shelter. The positions of food and water in the arena must ensure that the cockroaches are forced to come into contact with the product.
- Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed. 10 adult males, 10 adult females and 20 nymphs are included in each replicate.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).
- The observation of cockroaches should be conducted at different time points depending on the claim (e.g. after some hours, 1 day and up to 7 days post-exposure).
- Product application should be done outside of the test room.

Different methods of monitoring the cockroaches and assessing the repellent effect of the product are possible, e.g. by using a photo or video observation system or by applying adhesive material to the floor of the arena.

The adhesiveness of adhesive material varies and depends on the cockroach species, and it must be excluded that cockroaches are repelled by this material. Therefore, the catching efficacy of the adhesive material must be demonstrated for each cockroach species. At least 80% of cockroaches should be caught by the adhesive material. This can be demonstrated e.g. by catching of at least 80% of the test individuals in the control.

Proof of non-insecticidal effects: The mortality of the cockroaches can be monitored at the end of each trial, if no adhesive material is used. Alternatively, the same test set-up can be used without adhesive material to monitor the mortality of the cockroaches at the time points used in the repellent efficacy tests (day(s) post exposure).

Efficacy assessment: The potential repellent effect of the product is determined by comparing the number of cockroaches on the area protected with the repellent product to the number of cockroaches on the same area in the negative control.

5.6.5.5.2.2.1.2 Field trial

In the field trials the product is tested in an actual use situation, for instance in an infested home or warehouse and applied according to the directions for use on the label and in the SPC and against the claimed species (see 5.6.5.5.2.1). An example of an appropriate study design, e.g. number of replicates, observation parameters for a field trial in an infested area including a description of a pre-test to determine the initial population size can be found in Appendix 19.

Non-insecticidal efficacy, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8) should be examined at the end of the trial, if possible. In the case it is not possible mortality has to be evaluated in a simulated-use test.

5.6.5.5.2.2.2 Products intended for use as spatial repellents

For product authorisation purposes the efficacy of spatial repellents should be proven in:

- a simulated-use test according to the instructions of use (test design example see 5.6.5.5.2.2.2.1), or
- a field trial according to the instruction for use (test design example see 5.6.5.5.2.2.2.2).

Only products that affect the target organisms by application of the product in the air and not by walking on treated surfaces can be claimed as spatial repellents (for more details see Introduction, section 5.6.5.1.4).

The repellents used for the test should be identical to the product to be marketed.

The room size stated in the SPC and on the label must be used in the efficacy trial. For the extrapolation to larger room sizes than proven in the efficacy test a justification is necessary.

For a general claim "spatial repellents" the repellent effect must be proven. For the specific claim "dispelling" the dispelling effect must be proven.

5.6.5.5.2.2.2.1 Simulated-use test

Mandatory requirements:

- The simulated-use test that evaluates the repellent efficacy of products intended for use as spatial repellents has to be performed in test chambers with a volume adapted to the claim stated in the SPC (at least 20 m^3).
- For ambient repellents for protection of large rooms, e.g. diffusers to protect rooms with a defined volume, a double room test should be performed, with at least one 20 m^3 test room for product application. Such spatial repellent products can repel cockroaches to protect rooms from entering and/or dispel cockroaches from already infested areas.
- Both test rooms/boxes contain water, food and a shelter.
- Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed. Each replicate has to use 10 adult males, 10 adult females and 20 nymphs.
- Product and negative control are separately tested. The negative control should be conducted with the same set-up as the treatment without the spatial repellent.
- Environmental conditions must be specified for the test itself (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range of 19–29°C. When efficacy at high temperatures is claimed, 40°C should be the test temperature.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

The following test design example could be used for observing both effects (repelling and dispelling). Other test designs than the following example can be accepted if the protocol is scientifically valid.

General set-up

The repellent product is applied in a test room of at least 20 m^3, which is adjacent to a second room of the same or different volume. In the test room, a box (at least 0.5 m^2) is placed in the corner furthest away from the repellent product. This box is connected on the shorter side via a tube (passage) with a second box (at least 0.5 m^2) in the adjacent room. The inner walls of both boxes are covered with talcum to prevent cockroaches from escaping. A shelter is placed in both boxes opposite the passage entry. Alternatively, the test can be conducted in two connected test rooms in which the cockroaches can freely move.

For recording, of the cockroaches at certain times photo or video observation systems above both boxes could be used to avoid disturbing the insects.

After product activation and opening of the passage, the light is turned off and the system should be exposed to a twilight/darkness rhythm of about 12 hours, undisturbed over the entire test period (only for evaluation purposes the experimental chamber should be entered with low lighting).

Repellent effect

The repellent product is applied and activated according to the instructions for use in the test room. In each replicate, a group of cockroaches is introduced in the box in the untreated room. During the acclimatisation period of at least 2 hours the entry of the passage tube is blocked. Afterward the passage is opened, and the cockroaches can move freely between both boxes/rooms.

Dispelling effect

In each replicate, a group of cockroaches is introduced in the box in the test room. During the acclimatisation period of at least 2 hours, the entry of the passage tube is blocked. After acclimatisation the dispelling product is applied and activated according to the instructions for use in the test room and the entry is opened. During the test period, the cockroaches can move freely between both boxes/rooms.

Evaluation for both test set-ups (repellent or dispelling effect)

The number of cockroaches in each box/room should be recorded at different time points depending on the claim, e.g. after some hours, 1 day or up to 7 days post-exposure. Cockroaches are exposed to the test set-up at several time intervals after product application including the end of the claimed residual period. The number of cockroaches in each box/room should be recorded for all time intervals at the same time post-exposure, e.g. 1-day post-exposure.

Efficacy assessment: The potential repellent effect of the product is determined by comparing the number of cockroaches in the box/room with the repellent product to the number of cockroaches in the room outside the product application.

In a control test without product application side effects of the test rooms should be excluded and unimpeded passage through the rooms must be demonstrated for each cockroach species that is observed in the test system. In the control test, the cockroaches must be equally distributed in both test rooms/boxes, as evidenced by non-significant differences between the two rooms/boxes.

5.6.5.5.2.2.2 Field trial

In the field trials the product is tested in an actual use situation, for instance in an infested home or warehouse and applied according to the directions for use on the label and in the SPC. An example of an appropriate study design, e.g. number of replicates, observation parameters for a field trial in an infested area including a description of a pre-test to determine the initial population size can be found in Appendix 19.

Proof of non-insecticidal effects, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8) should be examined at the end of the trial, if possible. In the case it is not possible mortality has to be evaluated in a simulated-use test.

5.6.5.5.2.2.3 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractants in traps should be proven in:

- a simulated-use test according to the instruction for use, or
- a field trial according to the instruction for use.

Any attractant should be tested against a negative control.

The attractants, e.g. the traps, used for the test should be as similar as possible to the product to be marketed (for details see Introduction, section 5.6.5.1.3.7). Any bycatch of non-target insects has to be recorded and identified at least to order, preferably to the family level.

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or attractant product (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range of 19–29°C. When efficacy at high temperatures is claimed, 40°C should be the test temperature.

If outdoor use is claimed, the test should be performed outdoors and the attractant product and control have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed. For a simulated-use test each replicate has to include 10 adult males, 10 adult females and 20 nymphs.

5.6.5.5.2.2.4 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.5.3 Assessment of authorisation

5.6.5.5.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for cockroaches in the following way:

Products intended for use as repellent products as surface treatment or spatial treatment

Non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

The required results for the different tests are:

Laboratory test or simulated-use test:

- ≥ 80% repellence or/and dispellence (depending on the claim) compared to the negative control is demonstrated within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period

Field trial:

- ≥ 80% repellence or/and dispellence relative to either control sites or pre-treatment levels within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period

Products intended for use as attractants without PT18 active substances

The required results for laboratory test, simulated-use tests, or field trials are:

- at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period; ≥ 80% of the test individuals trapped within the test period compared to the negative control (or according to the claim), from the beginning and until the end of the claimed efficacy period against the claimed species.

5.6.5.6 Fleas

5.6.5.6.1 Introduction

Fleas are small flightless insects that survive as external or epidermic parasites of mammals and birds. They are vectors of different zoonotic diseases, such as plague, murine typhus, cat scratch fever and allergic dermatitis triggered by flea saliva also with implications for humans. The cat flea *Ctenocephalides felis* is the most important ectoparasite of domestic cats and dogs worldwide.

The Oriental rat flea *Xenopsylla cheopis*, also known as the tropical rat flea, is a parasite of rodents and humans, and is a primary vector for bubonic plague and murine typhus. The human flea *Pulex irritans* is a cosmopolitan flea species that has a wide host spectrum. It can also be an intermediate host for the flea tapeworm cestode *Dipylidium caninum*. *Tunga penetrans* (also known as chigoe flea or jigger) is a parasite of mammals (dogs and humans) in most tropical and sub-tropical climates causing an inflammatory skin disease (tungiasis).

5.6.5.6.1.1 Biology

Of the over 2000 species of fleas (Siphonaptera), the cat flea *C. felis* and the dog flea *C. canis* are the most common in-home pests in the EU.

Fleas undergo complete metamorphosis (egg, larva, pupa, adult) and the lifecycle begins when an adult female finds a suitable host. Once found, the female flea remains on this host for the rest of its life. Females produce several eggs after each blood meal and can produce several hundred eggs in their lifetime. The laid eggs fall off the animal host and develop in the areas where the host spends its time. The eggs tend to accumulate in the lowest areas such as deep in fibres of carpets, in cracks and crevices in the floor, furniture, and furnishings or behind mouldings.

Larvae require high protein food for their survival. By feeding on the dry faeces of adult fleas, larvae receive the protein-rich food. The adult flea takes in more blood from the host than necessary for nourishment and excretes the remaining blood in almost pure form. Once dried,

the faeces fall off the host where the larvae can feed. The larvae spin a cocoon including the surrounding material and begin the pupal state.

During the pupal stage, the development can be delayed if the environmental conditions are unfavourable. The pupae can enter into the rest of several months (diapause). An adult flea emerges from the pupae after stimulation by external cues that indicate a host in the close surrounding. After emergence, a flea must usually find a host (located using visual and thermal cues) within a week, or it risks death due to desiccation.

Complete development from egg to adult occurs in as little as two weeks but can take much longer depending on environmental conditions.

The appearance and parasitic behaviour of *Tunga penetrans* differ: it is the smallest known flea, measuring 1 mm. After a bloodmeal, males are still mobile like other fleas, but the female flea burrows head-first into the host's skin, leaving the caudal tip of its abdomen visible through an orifice in a skin lesion. This orifice allows the flea to breathe, defecate, mate and expel eggs while feeding from blood vessels. The flea's abdomen swells with eggs later in the cycle, reaching a size up to 1 cm.

5.6.5.6.2 Dossier requirements

General dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

5.6.5.6.2.1 Test species

For a general claim "against fleas", the product should normally be tested on adult cat fleas *C. felis* or adult dog fleas *C. canis*. If products are exclusively claimed against larvae than the efficacy should be demonstrated for that developmental stage instead of adults. For specific species claims, tests should be performed with the claimed flea species. Test species should also be specific or known to be an ectoparasite of the host to be protected, e.g. *C. canis* for a product used for dogs, notably in case of topical products.

Due to the specificity of certain active substances, e.g. pheromones, for products based on an active substance with a species-specific mode of action, only effects against flea species that have been tested under simulated-use and/or field conditions, depending on the type of claim, should be claimed on the product label and in the SPC.

For insects from laboratory rearing used in the efficacy studies, age and feeding conditions should be reported (for more details see Introduction, section 5.6.5.1.3).

5.6.5.6.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.6.2.2.1 Products intended for use as topical repellents for human skin or clothing

For product authorisation purposes the efficacy of repellent products targeting the use on human skin and clothing should be proven in:

- a simulated-use test according to the instructions for use (test design example see 5.6.5.6.2.2.1.2), or
- a field trial according to the instructions for use (test design example see 5.6.5.6.2.2.1.3).

The repellent products used for the test should be identical to the product to be marketed.

Proposed label claims regarding the performance of the product must be simulated (for details see Introduction, section 5.6.5.1.3.5).

Proposed label claims regarding the protection of a specific type of fabric must be simulated, i.e. the same type of fabric has to be used in the simulated-use test.

5.6.5.6.2.2.1.1 Laboratory tests

Mandatory requirements:

- A standardized laboratory in-vitro assay should be performed.
- Fabrics or clothing should be treated at the recommended application rate(s) according to the label claim and SPC.
- Replicates: A minimum of 5 independent replicates (each replicate contains 10 individually tested fleas) should be performed (each treatment and negative control).
- Environmental conditions must be specified for the test itself and the drying of the product, and during storage of the treated fabrics or clothing (temperature, humidity and photoperiod). The temperature of the test room would be expected to fall in the range between 20-23°C.

Other test designs than the following examples can be accepted if the protocol is scientifically valid.

Warm Object Bioassay (see Appendix 18: Büchel K., Kleier S., Dautel H.)

The "Warm Object Bioassay" is suitable for the determination of repellency of liquid formulations applied on fabrics/clothing and treated fabrics. The test should determine, if a flea is repelled by treated fabrics in the presence of a host mimic (heat source). The experiment exploits that adult fleas are attracted to warm objects corresponding to the host body temperature and are negatively geotactic.

Test design: In the middle of a test arena (at least 900 cm²) a warm object is placed simulated by a narrow glass cylinder (high: > 20 cm) containing warm liquid (35–36°C) as heat source, so that the surface temperature of the cylinder corresponds to the external body temperature of the host. The surface of the cylinder is covered by filter paper or test fabric (for products applied on or incorporated in textiles). The fabric used has to be representative of the fabrics commercially used, e.g. thick uniform cotton for military clothing. The repellent product should be applied at the recommended application rate(s) following the product use instructions on the test fabric or clothing and must be dry before conducting the test. Each centimetre a horizonal line is drawn on the fabric/filter paper to determine the distance movement of the fleas on the object as an additional behavioural parameter.

In total at least 50 unfed adult fleas should be tested for each test product. After testing of 10 fleas (= one replicate) the surface (test fabric/clothing) should be removed and replaced by a new one (newly sprayed). The total observation time for each individual is a maximum of 4 minutes.

A flea is not repelled when it, within the 4 minutes observation time, either

- climbs or jumps onto the test fabric/clothing and remains on the treated surface for more than 2 minutes, or
- reaches the upper end of the warm object within 2 minutes.

In all other cases, the fleas are considered as repelled.

It is therefore possible to test contact repellents as well as repellents acting over short distance.

Efficacy assessment: The repellent effect is evaluated by the time and distance fleas stay or crawl on the "warm object". Total vertical distance movement can be recorded in centimetre steps.

Blood Feeding Assay

The following test method is a suitable set-up for the determination of the repellency of liquid formulations.

Test design: The Blood Feeding Assay consists of an apparatus used basically for artificial feeding for adult cat fleas, see Appendix 18: Wade, S.E., Georgi, J. R.. The automated, temperature-controlled feeding unit is covered with a membrane. The unit is subsequently filled with bovine blood and set at 37°C.

10 unfed adult fleas are transferred into a plexiglass ring (approximately 1.5 cm height and 4 cm diameter) that is covered with nylon gauze on both sides. Before covering the plexiglass ring with the fleas, the upper side of the nylon gauze is treated with product at the recommended application rate(s) following the product use instructions. After product

application and the completely drying of the nylon gauze the feeding unit is placed on the treated nylon gauze, allowing the fleas to feed through the gauze and the membrane for 1 hour. A Petri dish with filter paper is placed under the caged fleas to evaluate the extent of feeding by flea faeces or blood traces caused by accumulating on the filter paper in the course of feeding.

Efficacy assessment: The repellent effect is determined by comparing the proportion of feeding between treatment and control. The proportion of feeding is quantified over the area of flea droppings caused through blood-feeding in mm^2 on the filter paper below the plexiglass ring. Alternatively, the proportion of feeding could be determined by evaluating the number of engorged fleas.

5.6.5.6.2.2.1.2 Simulated-use test

Mandatory requirements:

- The efficacy of repellent products applied on human skin or clothes according to the instructions for use should be proven in a simulated-use test. A test set-up similar to an "arm-in-cage" or "arm-to-cage" test would be suitable; a negative control should be included.
- Efficacy data of at least 10 different volunteers (preferably volunteers with different hairiness of the arms, different genders; age: 18 - 65 years) should be collected since repellence/attractiveness to fleas varies considerably between human individuals. For efficacy assessment, valid data of at least 10 different volunteers must be used.
- Repellent products have to be applied at the recommended label dose.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

5.6.5.6.2.2.1.2.1 Volunteers

Volunteers' attractiveness is measured by exposing the untreated skin to caged populations of host-seeking, pathogen-free, unfed fleas, and the results must be presented in the report. A minimum of 5 probings per minute must be achieved. To avoid unnecessary blood feeding, the volunteers are allowed to softly brush off the fleas after probing with a soft brush. In case probing activity is lower, a new cage with fresh fleas should be used or additional fleas could be added and reported.

Volunteers need to be fully informed about the aim, the procedure and the expected duration of a study. The study should be carried out in compliance with the national ethics regulation. They also need to be informed about potential side effects such as allergic skin reactions caused by the product. Their participation is voluntary and can be recalled at any time before and during the study, see Appendix 18: WHO and EPA guidelines. 12 hours before and during testing, volunteers should avoid nicotine, alcohol, fragrance (perfumes, body lotions, soap, etc.) and repellent products. Any side effect observed during the test should be mentioned in the test report.

Prior to the application of the repellent, the skin is washed with fragrance-free soap and rinsed with water. The hand should be covered with a glove that fleas cannot bite through during each exposure to the test insects. If the entire forearm is to be treated with repellent, the skin area should be estimated according to recommendations of the WHO: the mean circumference is calculated by measuring the circumference at the wrist and elbow and multiplied with the length of the arm (between wrist and elbow). Alternatively, an "arm-to-cage" test can be conducted, i.e. repellents can be applied to a defined area on the forearm, e.g. a 100 cm^2 window. In "arm-in-cage" tests, the rest of the arm will then be protected from bites by a custom-made sleeve (flexible material that prevents biting, edges are not treated with the product; contamination should be prevented). The use of a defined area adds to a standardisation of test conditions; therefore, the use of a sleeve is recommended.

5.6.5.6.2.2.1.2.2 Efficacy testing

Other test designs than the following examples can be accepted if the protocol is scientifically valid.

A rectangular gauze cage (at least 27 L) with an opening for the arm on one side and fleas on the other side in a given harbourage should be used. For acclimatization fleas should be introduced in the cage 1 hour before the test.

Prior to the efficacy test of the treated arm, the probing pressure (5 probings/minute) of the pathogen-free, unfed test fleas needs to be verified with the other, untreated control arm. The treated skin should be exposed in regular intervals, e.g. once an hour.

Efficacy assessment: While testing for repellence, an endpoint for the failure of repellence for subjects treated with the recommended label dose should be selected. Efficacy failure in a test to determine CPT is the time from application of a repellent until efficacy failure by a confirmed event (definition see Introduction, section 5.6.5.1.5). Repellent efficacy should be based on the median or mean CPT (CPT calculation, see Introduction, section 5.6.5.1.5).

Proof of non-insecticidal efficacy: After the test fleas should be kept under optimal rearing condition to observe whether insecticidal effects are seen after a contact/exposure to the test repellent product. Mortality of the insects should be monitored after 24 hours.

5.6.5.6.2.2.1.3 Field trials

Field trials allow the evaluation of topical repellents against wild flea populations under realistic conditions at the recommended label dose. In contrast to simulated-use tests they are, however, subjected to greater variation, regarding probing activity, flea species, population density, temperature, humidity, light conditions, etc. Probing pressure on untreated skin should be at least five fleas within one minute. Volunteers (at least 10; preferably volunteers with different hairiness of the arms, different genders; age: 18 - 65 years - for more details see 5.6.5.6.2.2.1.2.1) trained on the use of an aspirator can serve as their own control on the other, untreated, arm or leg. Alternatively, 10 untreated volunteers could be included in the field trial, as control. The forearm (between wrist and elbow) or lower leg (between knee and ankle) serves as the treatment area. The skin area should be measured and treated as described under 5.6.5.6.2.2.1.2.1. Product application must follow the claimed instruction of use. The treated skin will be exposed in regular intervals. Frequency of assessments will be not less than every hour until CPT ends (occurrence of the first confirmed probing, see Introduction, section 5.6.5.1.5). Exposure periods should be synchronized with the time when the relevant species are abundant. The treatment of arms or legs should also be consistent with the feeding preference of the target species. Time of day at which volunteers are treated and at which exposure started and ended should be reported. Fleas that probe or bite during an efficacy test can be collected, where possible, for later identification. Before the study is conducted, the presence of the relevant flea species has to be verified. This should be documented in the study report.

5.6.5.6.2.2.2 Products intended for use as topical repellents on animals or animal clothing

For product authorisation purposes the efficacy of repellent products targeting the use on animal skin or animal clothing should be proven in:

- a simulated-use test according to the instructions for use (test design example see 5.6.5.6.2.2.2.2), or
- a field trial according to the instructions for use (test design example see 5.6.5.6.2.2.2.3).

Proposed label claims regarding the performance of the product should be simulated in the study. For the claim “unaffected by washing”, the label and the SPC must indicate how often the animal can be washed without reducing the efficacy of the biocidal product. In the efficacy test, the test individuals should be repeatedly washed with a non-insecticidal, fragrance-free shampoo during the trial according to the number of wash cycles indicated. If a product claim is to protect the entire animal, this should be demonstrated (notably for collars). For specific claims regarding products applied on clothes or fabrics see Introduction, section 5.6.5.1.3.5.

The efficacy data should be relevant to prove the submitted claims. Therefore, the efficacy of biocidal products that are limited to a body area, e.g. collars for animals must be proven for the whole test individual that should be protected. If only specific body areas intend to be protected, e.g. ears, udders, etc. then the efficacy for the claimed area must be proven.

For specific claims (prevention of bites through/prevention of bites next to the treated clothes), relevant tests have to be submitted.

5.6.5.6.2.2.2.1 Laboratory test

A standardized laboratory test, e.g. "Warm Object Bioassay" (see Appendix 18: Büchel K., Kleier S., Dautel H.) can be used to observe the behavioural response of fleas to a treated surface (for a details see 5.6.5.6.2.2.1.1).

5.6.5.6.2.2.2.2 Simulated-use test

Repellents for use on animals, e.g. dogs, cats should be tested in a simulated-use test. The repellent effect should be demonstrated for each target animal species claimed. Dogs should be used as a substitute for cats, as testing with cats can imply certain animal welfare issues. The repellent product is applied according to the instructions for use. If the product is acting at very close range, it may be possible to make the evaluation by treating parts of the host, like one treated side of the neck compared with the equivalent area on the other, untreated, side of the neck. The advantage of such a setup is the neutralisation of individual host differences in flea-attraction. The control body part should resemble the treated body part, e.g. front-half vs back-half or left side vs right side of the animal. In case of a restricted application, e.g. collar, a spot-on product with a claim of entire body protection, or for products acting in a wider range over the entire body the test should permit to validate the efficacy of this kind of application by testing the entire animal. In this case, untreated animals must be used as a negative control.

Test animals: It is recommended to include at least 10 animals per treatment/control group of a different breed and sex, i.e. for testing on entire animals at least 10 treated and 10 negative control individuals are necessary. The age, hair/fur length, weight and coat colour of each animal should be recorded. It is necessary that animals come from suitable, e.g. non-smoking households. Every side effect observed during the test should be mentioned in the test report. 24 hours before and during testing, animals should not be treated with fragrance (perfumes, soap, etc.). The test animal must be free of fleas and should not be protected by any repellent/insecticidal residuals caused by previous repellent and/or insecticidal treatment (veterinary medicinal product). Therefore, the animals shall be screened and cleaned before the repellent is applied. Concerning endo-antiparasitic agents this is acceptable, but details (product, dose, application rate, application method, date of the last treatment, and residual efficacy) must be given in the report and the negative controls and the treated groups must have the same status. A safety margin for externally applied anthelmintics is 4 weeks after the end of the last application. The history of the treated and the control groups should be comparable. The treatment status of the animals should be specified and only animals for which the insecticidal/repellent effect can be excluded should be included in the study.

Proof of non-insecticidal efficacy, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8): After the test fleas are maintained in a test system to observe whether insecticidal effects are seen after exposure to the test repellent product. Mortality of the insects should be monitored after 24 hours.

5.6.5.6.2.2.2.3 Field trials

For PT19 products which are applied on animals, no common protocols are available. However, several guidelines for the evaluation of ectoparasiticides are available, please see Appendix 18. These guidelines can be adapted for the demonstration of the efficacy of repellent products to be applied on animals. If field trials are conducted, they must take place in an area with an appropriate flea density. These tests should preferably take place in Europe. Where tests in Europe are not possible, the conditions and flea species must be confirmed, and their relevance justified.

The repellent effect should be demonstrated for each target animal species, e.g. dog, horse, etc. claimed. Dogs should be used as a substitute for cats, as testing with cats can imply certain animal welfare issues.

Test animals: For details see 5.6.5.6.2.2.2.2.

The investigator is reminded that the validity of the results is directly related to the degree of variability in the test. Increasing the number of test animals could increase the reliability of the test results, see Appendix 18: OPPTS 810.3300.

The product should be applied according to the claim.

Depending on the animal species, several factors, e.g. hair length, thickness of the coat, self-grooming, etc. might impact the efficacy. These factors should be taken into account in the demonstration of the efficacy. The two groups should be kept separately, to avoid contact of the control animals with the treated fur.

Claimed application rates should take into account the type and weight of the animals.

Proof of non-insecticidal efficacy, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8): During the trial fleas should be collected and after the trial maintained in a test system to observe whether insecticidal effects are seen after exposure to the test repellent product. Mortality of the insects should be monitored after 24 hours.

5.6.5.6.2.2.3 Repellent products intended for use as surface treatment

For product authorisation purposes the efficacy of repellent products should be proven in:

- a simulated-use test according to the instruction for use (test design example see 5.6.5.6.2.2.3.2), or
- a field trial according to the instruction for use and additionally a laboratory trial (test design examples see 5.6.5.6.2.2.3.1) testing the required different surfaces.

Products applied onto surfaces may act either by evaporation or on the surface itself.

For products applied on surfaces, two porous and one non-porous surface should be used, e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete, for a general label claim as "surface treatment". The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces. For authorisation of a product to be used on a specific type of surface the efficacy for only this specific surface should be assessed. If a specific surface type is claimed, e.g. dog mattress, this surface has to be tested.

The repellents used for the test should be identical to the product to be marketed.

For residual efficacy, fleas are exposed to the product at several time intervals after application (including the end of the claimed period).

Proposed label claims regarding the performance of the product should be simulated in the study. For example, for the claim "unaffected by cleaning/vacuuming", the surface should be repeatedly cleaned during the trial (for details see Introduction, section 5.6.5.1.3).

5.6.5.6.2.2.3.1 Laboratory test

A laboratory test can be performed to observe the behavioural response of fleas to treated surfaces, e.g. plywood, carpet, ceramic tile, painted plywood, stainless steel, concrete. Other test designs than the following example can be accepted if the protocol is scientifically valid.

Test design: A box (at least 120 cm^2) covered with gauze for ventilation serves as test arena. The relevant surfaces have to be applied with the repellent product at the recommended application rate(s) following the product use instructions. Half of the arena is covered with the treated and the other half with the untreated surface. An attractant source should be placed on the treated area. The negative control (e.g. entire arena covered with untreated surfaces) runs under the same conditions.

10 unfed adult fleas per replicate are released on the untreated side of the arena.

Replicates: A minimum of 5 independent replicates should be performed (each treatment and negative control).

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation). Conditions during the test have to be maintained at 25°C±2°C and relative humidity of > 75%. Conditions during the storage have to be maintained at 20-25°C and a natural humidity unless specific conditions are required according to the claim.

Efficacy assessment: The number of fleas on the treated and untreated surface should be recorded. The distribution of the fleas in the control treatment replicates has to be non-significantly different.

5.6.5.6.2.2.3.2 Simulated-use test

Mandatory requirements:

- Replicates: A minimum of 5 independent replicates should be performed (each treatment and negative control). Each replicate consists of at least 10 unfed adult fleas.
- Test conducted in an arena with a minimum size of 0.5 m^2.
- Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range of 20-25°C.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).
- For a claim "prevents biting", testing needs to be conducted with volunteers, depending on the claim either humans or animals, in order to determine the CPT (CPT definitions and calculation see Introduction, section 5.6.5.1.5). For efficacy assessment, valid data of at least 10 different volunteers (humans or animals) must be used.

The following choice-test could be used for observing the repellent effect on surfaces. Other test designs than the following example can be used if the protocol is scientifically valid.

Test design: In a ventilated test room two identical types of surface, e.g. dog basket at a distance of 1 m should be used at the same time. The repellent product is applied at the recommended application rate(s) following the product use instructions on one surface, whereas the second untreated surface represents the control. For easy detection of fleas, the surface should be of light colour. To lure the free-ranging fleas to the surfaces an attractant source and optionally a heat source, e.g. a heating mat, are used to simulate the body temperature of the host (35-37°C).

10 unfed adult fleas mixed sexes per replicate are released in the middle of the test room equal distance between both surfaces and can move freely within the room. Access to the surfaces should be barrier-free and crawling under the surfaces should be inhibited. During the test-run, the number of fleas should be monitored at several time intervals after product application (including the day of treatment and at the end of the claimed residual period) on both surfaces e.g. by high-resolution action cams installed above.

Efficacy assessment: The repellent effect (%) should be calculated relatively to the negative control surface. The number of fleas on the treated and the negative control surface should be recorded. Individuals who are neither on the test surface nor on the control surface should be not included in the evaluation.

5.6.5.6.2.2.4 Repellent products intended for use as spatial repellents

For product authorisation purposes the efficacy of spatial repellents should be proven in a simulated-use test according to the instructions for use.

The repellents used for the test should be identical to the product to be marketed.

When the label claim says that the product should be used in ventilated rooms the opening of windows and doors should be simulated in the test.

The room size stated in the SPC and on the label must be used in the efficacy trial. For the extrapolation to larger room sizes than proven in the efficacy test a justification is necessary.

For a general claim "spatial repellents" the repellent effect must be proven. For a specific claim "dispelling" the dispelling effect must be proven.

If a claim states that the product prevents insects from biting/probing, a simulated-use test proving the biting/probing inhibition with a volunteer in the room, depending on the claim either humans or animals, is necessary.

In case of a specific claim regarding the use in specific climate conditions, e.g. high temperature or tropical conditions the efficacy must be proven under these conditions.

Replicates: A minimum of 5 independent replicates should be performed (each treatment and negative control).

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation).

Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

5.6.5.6.2.2.5 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractants in traps should be proven in:

- a simulated-use test according to the instructions for use, or
- a field trial according to the instructions for use.

Any attractant should be tested against an untreated control.

The attractants, e.g. the traps, used for the test should be as similar as possible to the product to be marketed (for details see Introduction, section 5.6.5.1.3.7). Any bycatch of non-target insects has to be recorded and identified at least to order, preferably to the family level.

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or attractant product (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range of 20-25°C.

If outdoor use is claimed, the test should be performed outdoors and the attractant product and control have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for detailed information regarding the requirements see Introduction, section 5.6.5.1.4.1.3).

Replicates: A minimum of 5 independent replicates should be performed (each treatment and negative control).

5.6.5.6.2.2.6 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.6.3 Assessment of authorisation

5.6.5.6.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for fleas in the following way:

Products intended for use as repellent for human or animal skin, clothing:

Non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

The required results for the different tests are:

Laboratory test (not required for product authorisation):

- 100% repellency within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

Simulated-use test and field trial:

- during the claimed protection period complete protection should be proven expressed as mean or median CPT (for details see Introduction, section 5.6.5.1.5).

Products intended for use as general surface and spatial repellents:

Possible insecticidal effects have to be examined in a simulated-use test (for details see Introduction, section 5.6.5.1.3.8).

The required results for laboratory test (not required for product authorisation of spatial repellents), simulated-use test and field trials are:

- ≥ 80% repellency within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

If "prevention of bites" is claimed, then 100% repellency (CPT) is also required for surface and spatial repellents for the claimed period.

Products intended for use as attractants without PT18 active substances:

The required results in the simulated-use test are:

- at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period;
- ≥ 80% of the test individuals trapped within the test period compared to the negative control within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

5.6.5.7 Flies on grazing cattle, horses and other livestock

5.6.5.7.1 Introduction

The fly-fauna on cattle, horses and other livestock is complex and many of the species have different impacts on their hosts. Flies on grazing livestock in pastures represent a very significant problem. These flies are divided into two groups, the biting flies and the sucking flies. Biting flies are penetrating the skin of the host to suck blood, while they provoke irritated reactions from the hosts. When biting flies are present in high numbers it may affect health as well as productivity. Sucking flies feed on sweat, tears, saliva, urine, faeces, exudates and blood from wounds. Sucking flies are in constant movement between all sorts of exudates and thereby may transmit a variety of diseases. They cannot penetrate the skin, with their proboscis, but many species have enlarged prestomal teeth in their oral aperture which can enlarge or even reopen nearly healed wounds.

PT19 biocides against flies on grazing cattle, horses and other livestock can only claim to repel or attract (in order to trap) the target organism, not to prevent the diseases.

5.6.5.7.1.1 Biology

Hydrotaea spp. are sucking flies, often to be seen in the eye region, around the udder, and around both new and nearly healed lesions where they seek all sorts of exudates. *Hydrotaea* cannot penetrate the skin but have prestomal teeth on labellum which can enlarge wounds. *Hydrotaea irritans* (head fly) is an important fly from an animal health point of view. It deposits its eggs in the ground in hedges and scrubs with high grass. The larvae are carnivorous, eating other insect larvae in the ground. It normally has one generation per year. The larvae overwinter and pupate in late winter and hatch into adult flies in late June. Other important species of sucking flies are *Morellia* spp., *Musca domestica*, *Musca autumnalis,* which develop directly in cattle-manure and have several generations per year. These sucking flies must be seriously considered when repellence is claimed as they all have the potential to transmit mechanically the diseases, e.g. *M. autumnalis* transmit parafilaria, and *Hy. irritans* transmit summer mastitis.

Several biting flies such as horn flies *Haematobia irritans* and *Haematobia stimulans* are very common on cattle. These species develop in cattle manure, which means that these biting flies are normally only seen on horses when the horses are grazing close to pastured cattle. They are both obligate blood-feeders, and primarily attack cattle and horses for a blood meal. They both have several generations during the summer. The horse fly *Hypobosca equina* is found in some areas mainly on horses, but also from time to time on cattle. The stable fly *Stomoxys calcitrans* also belong to the group of biting flies but breed in fermenting organic matter mixed with different kinds of manure and is, therefore, more common close to barns and cowsheds. A specific chapter is concerned with the stable fly indoor.

Tabanids (horse flies, deer flies and clegs) are undoubtedly a very important reason for using repellent on horses, and cattle can also be heavily affected by the painful bite of tabanids. The small blood leaking lesions they make are attracting sucking flies and these can even be seen trying to push the horseflies aside to get access to the leaking blood themselves. Big tabanids may only be seen in huge numbers for a short while and are therefore difficult to use as test species. The horse fly *Haematopota pluvialis* on the other hand is often very abundant throughout the summer in permanent and old grazing areas, which has neither been ploughed nor fertilised. The bite of *Tabanids* is painful and causes considerable disturbance to horses and cattle as well as to people working horses. All the above-mentioned flies live in close contact with their host and frequently land on them, which makes them vulnerable for treatment with repellents.

Apart from these species, there is a wide range of botflies which attack livestock. Botflies are obligate parasites and therefore always need a host for larval development and may cause serious damage as endoparasites. These are flies like horse bots (*Gasterophilus* spp.), sheep

bot fly (*Oestrus ovis*) or warble fly (*Hypoderma* spp.*)* which causes considerable disturbance among grazing animals, when they are present. The larvae of horse bots are endoparasites in horses while the ox warble fly is an endoparasite in cattle. The adult female flies of these botflies never get in contact with the horse coat as they spray eggs on the host's coat without landing. On horses, eggs are often found attached to the forelegs but can also be seen on the mane or even the flanks. Horses take the fly larva in when licking and the larva develops in tongue epithelium and later the stomach. The ox warble fly deposits its eggs on cattle (preferably on legs) and from there the larvae penetrate through the skin, develop in the connective tissue, and end up causing swellings called "warbles" on the skin surface. As the botflies do not land, it means that any repellence is difficult to measure and methods must be developed in each case.

One last important group of flies in this context is blowflies (*Calliphoridae*). The female of some of these species like *Lucilia* spp. seek carcasses for feeding and egg laying and the larvae develop in the carrion. Sometimes though, blowflies lay eggs in living tissue in cattle or horses and the larvae may develop in wounds or in body cavities. As these flies land on the hosts they may be liable to a repellent.

5.6.5.7.2 Dossier requirements

Dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

5.6.5.7.2.1 Test species

Few of these fly species can be kept in captivity, and probably none can act biologically relevant in captivity when sensitive biological parameters such as host-seeking are investigated. The important issue, when the efficacy of a repellent is measured, is whether a product, when applied to the host in a relevant environment, can overrule all the key stimuli that attract flies to their host. Tests with laboratory colonies of relevant species as well as with field collected specimens are applicable, provided such testing is justified in detail.

Products intended for use as a repellent on cattle, horses and small ruminants or spatial repellents should be tested using the claimed host. A general host claim is not acceptable.

Products intended for use as a repellent against specifically claimed fly species, e.g. a *Gasterophilus* species must be tested with the species claimed, and efficacy against this species must be demonstrated.

Spatial or topical repellent products intended as a general fly-repellent claimed to protect cattle, horses or other livestock against groups of nuisance flies (sucking flies, biting flies, tabanids, botflies, blowflies) must be tested with representative species for the claimed host. The relevance of target fly species per host animal should be appropriately justified. For cattle and/or horses, the following species may be suggested as they are relatively easy to identify, but local adjustments using other fly species are acceptable if well argued: Sucking flies of the genus *Hydrotaea, Musca* and *Morellia*, the biting flies *Haematobia irritans* (which can be omitted on horses, if the horses are grazing isolated from cattle), and the horse flies *Haematopota pluvialis*. In some areas, other tabanids are more relevant and it is acceptable to use them instead if it is well argued in the report.

A general claim for use on the claimed host animal can only be given if efficacy against all groups is proven.

In case of specific claims, e.g. effective at high temperature, in contact with water, when the hosts are sweating, when horses are at work, etc., efficacy must be demonstrated in the relevant situations.

It must be clearly described in the report how the test method ensured that the claimed fly species were counted in the field experiment.

5.6.5.7.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.7.2.2.1 Products intended for use as repellents

The efficacy of a product should be shown in either a robust simulated-use test, where animals can be tethered and observed close by or a field trial where the animals are loose/untethered and observed from a distance to establish the efficacy and persistence of the product when used according to the label in a real-life situation.

For PT19 products which are applied on animals, no common protocols are available. However, the EMA has published several guidelines for the evaluation of ectoparasiticides, see Appendix 18. These guidelines may be adapted for the demonstration of the efficacy of repellent products to be applied on animals, but any well documented protocol can be used, provided it is scientifically sound.

It is important to give a detailed description of the circumstances in which the experiments are conducted, with special emphasis on the ratio of treated to untreated host animals at each trial location and trial replicate. It may be of consequence for the trial outcome whether all hosts in a herd are treated, compared to a situation where only a minor part of the hosts in a herd are treated. It is also of importance that the daily variation of fly activity is taken into consideration in the setup of the efficacy trials and reported in detail. If the product is part of a push-pull strategy or a spatial repellent, e.g. hosts with repellent combined with attracting traps, then such a setup must be reported in detail as well.

If treated horse rugs or similar covers are claimed to be effective in the field, then it must be demonstrated effective in the field. It is not important to make an evaluation of the flies on the rug itself, but the emphasis must be on the exposed parts of the host such as the neck, head, legs and belly, including the sheath on males and udder on females, which are thin-skinned areas and very attractive to several blood-feeding species.

Tests must be presented with all host animal species claimed. Bridging efficacy data from one host species to another can be accepted based on robust scientific justification taking into account e.g. the difference in dosing between species (a small goat vs. a large cow), whether sweating is possible or not.

5.6.5.7.2.2.1.1 Laboratory test

Laboratory tests of repellents are not advised, as the differences in food seeking biology of the relevant species is huge.

5.6.5.7.2.2.1.2 Simulated-use test

In a simulated-use test different parameters are controlled. A simulated-use test of a repellent with full control of host animals and absence of their normal avoidance behaviour can be used to evaluate the efficacy and the persistency of the treatment; topically, spatial or impregnated textiles. The product should be applied according to the claim. Environmental conditions must be specified at the beginning and during the test (temperature, humidity, photoperiod). No specific temperature or humidity are recommended, but it must be warm and not too windy to have fly activity high enough to challenge the product. A control treatment without repellent should be included in all trials to secure a measure of fly activity.

It would be possible to do simulated-use tests e.g. an animal-to-cage method similar to arm-in-cage, with stable flies (*S. calcitrans*), house flies (*M. domestica*) and horn flies *(Ha. irritans*) as these can be kept in laboratories. Therefore, specific claims on these species may be backed by data generated in simulated-use experiments, but it will not be possible to extrapolate such data to cover any other species encountered on grazing livestock.

A simulated-use test must be conducted at a time in the season, as well as on days where the relevant fly species are active in the field. Depending on the target animal species, several factors, e.g. hair length, thickness of the coat, grooming, etc. might impact the efficacy of topical repellents. Explain in the test report/documentation the circumstances around the choosing of the test animals. If animals of different colour are chosen be sure not to have a majority of light-coloured animals. Ideally light coloured animals are not included, but state on the label if the product is for a specific colour of animals, if only light coloured animals are tested a sound justification is needed to include all fur colours in the claim. For products only tested on e.g. pied (black or red) cattle it can be necessary to state on the label that the product is for pied (black or red) cattle. For products tested on sheep it can be necessary to

test on both sheared and unsheared sheep (a sound justification is needed if only sheared or unsheared sheep are tested).

At least two groups of host animals must be used, one group treated with the product and one group with no treatment. At least ten different individuals are suggested to be used in each group. In the case of horses, a setup could be 2x5 horses with a treatment switch after e.g. a week (depended on the residual efficacy of the tested product) tested at one location, or 2x5 animals tested at two different locations. At the end of the test period, there must be 2x10 valid datasets. Each treatment group should be housed in separate enclosures throughout the course of the trials to prevent cross infestation or cross-contamination.

Preferably, no test animals should have a recent history of treatment with insecticide/acaricide or antiparasitic agents. Concerning endo-antiparasitic agents this is acceptable, but details (product, dose, application rate, application method, date of the last treatment, and residual efficacy) must then be given in the report; the controls and the treated groups must have the same status. A safety margin for externally applied anthelmintics is the end of last application +1 month. The history of the treated and the control groups should be comparable.

Livestock should be treated with the test product once the infestation has become established. The infestation should be documented by e.g. recordings of the numbers of flies, photographs. Dependent on the circumstances tests can be conducted using treated groups in comparison with untreated groups. If the product is acting at very close range, it may be possible to make the evaluation by treating parts of the host, like one treated side of the neck compared with the equivalent area on the other, untreated, side of the neck. The advantage of such a setup is the neutralisation of individual host differences in fly-attraction. For dose determination, it may be preferable to use tethered hosts. The attractiveness of the test animals or experimental groups should be tested prior to treatment.

Percent inhibition (landing or feeding) is based on the number of flies landing/feeding on the untreated control animals (or room) and the number of flies landing/feeding on the treated animals (room):

Pct inhibition: $((\text{LandingFeeding}_{\text{Control}} - \text{LandingFeeding}_{\text{Treated}})/\text{LandingFeeding}_{\text{Control}}) \times 100$.

Alternatively or in addition to fly landing catches, also the frequency of certain horse's avoidance behaviour according to Mottet et al., see Appendix 18, can be recorded as a substitute, provided the abundance of the target species predominantly causing the avoidance behaviour is measured at the beginning and the end of the test. Test designs may also be adapted from Japin, M. and Haanen, G. A. Y., see Appendix 18.

5.6.5.7.2.2.1.3 Field trials

In a field trial, the animals must have the opportunity to display normal avoidance behaviour considering how the product is intended to be used. The tests may be conducted as described for the simulated-use test above, with the adjustment that there is no interference with the host animal's normal behaviour in the field.

The field trials should be conducted on two different geographical locations.

At least two groups of host animals must be used, one group treated with the product and one group with no treatment. At least ten different individuals are suggested to be used in each group. Due to feasibility and cost-effectiveness, it would be possible to use 10 animals one week (5 each in the treatment and control groups) and then use the same individuals again in the second week, but swap treatments. Each animal would then act as its own control. However, any residual activity of the product has to be excluded. The field trials can be performed twice with 5 animals (in each of the control and treatment groups) each time at the same location, or with 5 animals (in each of the control and treatment groups) at two different locations. At least 10 animals (each as control and treatment) must be tested. The two locations can be in the same country or region, e.g. two villages. In the case of cattle, the test can be performed at only one location using 10 cows for the control and 10 cows for the treatment

As documentation for persistency, counts are repeated with chosen intervals of lengths dependent on the claim. If there is a claim for use on wet/sweating hosts, then data must be provided showing that the product is effective under such circumstances. The guideline from

the EMA: "Guideline on specific efficacy requirements for ectoparasiticides in cattle", and Herholz et al., see Appendix 18, may be of inspiration for field trial setup.

A field experiment to identify the level of efficacy of a product intended to protect cattle, horses or other livestock against flies must cover the following issues:

Locality: Preferably low-lying areas with shelter. Important to establish double fencing between the herds to prevent contact between treated and untreated hosts. Preferably treated and control herds should be interchanged during a trial period to minimise differences between the two paddocks/fields.

Herds: Two individual experiments each constituting a treated and an untreated group. The choice of herds is dictated by the need of herds with neighbouring herds suitable as controls. Each group must constitute at least ten individuals. For horses a minimum of five individuals is acceptable.

Census: The fly activity may be measured through the summer, preferably on windless, hot days with high humidity. Prior to launching experiments monitoring of fly infestation should be performed. Observations of flies must be made for the time period relevant for the relevant fly species. The insect activity on the two groups of hosts (treated and untreated) must be counted within the frame of one hour, where environmental conditions are comparable for both groups. Each time the number of flies must be counted on the herd. Observations are made in five different areas on one side of the host's body: at the head, back, side, belly, and legs. Within each region, the number of flies belonging to one of the test species is recorded. The chosen test fly species are counted by direct observation. The observer must walk around the herd to minimize the influence on the recordings of heterogeneous fly distribution caused by sunlight or breeze. The frequency of measures is dependent on the claimed persistency of the product.

Insects: The following species may be suggested as they are relatively easy to identify, but local adjustments are acceptable if well argued: Sucking flies of the genus *Hydrotaea*, and of the genus *Morellia*, the biting flies *Haematobia irritans* (which can be omitted on horses, if the horses are grazing isolated from cattle), and the horse flies *Haematopota pluvialis.* In some areas, other *Tabanids* are more relevant and it is acceptable to use them instead if it is well argued in the report.

The presence of botflies must be recorded when observed, but they are not very abundant, and the figures cannot be used in statistics. If a claim on botflies is sought it may be possible to evaluate their activity by the number of eggs on the skin, but a method is not described for this, and therefore, if done, it must be explained in explicit detail.

For a general claim against groups of flies (sucking flies, biting flies, Tabanids, botflies or blowflies), the species relevant for the claimed group (on the claimed host) must be present in sufficient numbers to be able to make a meaningful and statistically significant distinction between treated and control groups. If efficacy has been shown against all groups a general claim for use on the claimed host animal can be made. For a specific claim, all (individually) claimed fly species must be present in sufficient numbers.

5.6.5.7.2.2.2 Attractants without PT18 active substances

Use of an attractant in a trap in a push-pull setup may be suggested. A trap could be loaded with CO_2, heat, octenol, butyric acid, black colour ball, etc. In such a setup the fly load will be measured as described above.

For product authorisation purposes, the efficacy of an attractant in a trap without an active substance according to PT18 should be proven in a simulated-use test or a field trial according to the instructions for use.

The attractants, e.g. the traps, used for the test should be identical to the product to be marketed (see Introduction, section 5.6.5.1.3.7). Traps should be tested on their own, with a control tested separately in a similar test setting.

5.6.5.7.2.2.3 Attractants in combination with PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.7.3 Assessment of authorisation

5.6.5.7.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for flies on grazing cattle, horses and other livestock in the following way:

Products intended for use as repellent on animals (including a delivery system)

A simulated-use test or a field trial demonstrating ≥80% repellency within the test period (according to the claim), directly after product application and at the end of the claimed use period.

All claimed target host animals must be tested. Claims on herds as well as individuals are acceptable and should be tested as such.

In case of specific claims, e.g. effective at high temperature, in contact with water, when the hosts are sweating, when horses are at work, etc. these should be demonstrated.

Products intended for use as a spatial repellent or for use as attractants without PT18 active substances

A trap with an added attractant should catch significantly more flies than one without the attractant. The traps used for the test should be identical to the product to be marketed.

A simulated-use test or a field trial demonstrating ≥ 80% efficacy within the test period according to the claim, directly after product application and at the end of the claimed use period.

For attractants at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

All claimed target species must be tested.

The product label and SPC should state that the entire insect population might not be caught/repelled by this type of product; the label claim should be "reduces" and not "protects"/"protection", with no mention of a CPT.

5.6.5.8 Fruit Flies and Scuttle Flies

5.6.5.8.1 Introduction

The purpose of biocidal repellents or attractants for fruit flies and scuttle flies is to prevent and fight an infestation of food, domestic waste or medical and veterinary facilities.

Fruit flies may cause inconvenience primarily indoors where they infest rotting organic material such as fermenting fruits and vegetables.

In Europe, species of the family Drosophilidae (fruit flies or vinegar flies) can be considered as a relevant nuisance.

Fruit fly: *Drosophila* spp., e.g. *Drosophila melanogaster*.

Within the family Phoridae (scuttle flies, humpbacked flies or coffin flies), the introduced species *Megaselia scalaris* may cause inconvenience or hygienic problems primarily indoors while infesting food or wounds.

Scuttle fly: Phoridae, e.g. *Megaselia scalaris*

5.6.5.8.1.1 Biology

The life cycle of both fruit flies and scuttle flies contains four developmental stages: egg, larva (maggot), pupa and imago (adult). Most species of fruit flies have a saprophagous lifestyle, as both larvae and adults are feeding on decaying matter. Eggs are laid on rotting organic material including fermenting fruits and vegetables. Once hatched, larvae feed by burrowing into the organic debris and filtering decaying organic matter. They then undergo transformation into the imago via a pupal stage. Adults feed by regurgitating on food and then taking up the pre-digested food in liquid form. The life cycle of fruit flies, from egg to fly, is 1 to 3 weeks, depending on the climate conditions (shortest: 7 days at 29°C). The adult fruit flies have a life expectancy of about 40 to 50 days.

Fruit flies regularly fly into and out of man-made structures. Inside, fruit flies land on human food or organic waste. Fruit flies play only a minor role in spreading pathogens since they normally are not in contact with decaying or infective animal products, e.g. flesh, carcasses, faeces. They, therefore, do not pose a serious health risk; however, severe infestations can render the food unfit for human consumption, due to a common averseness against infested food and due to acceleration of fruit decay.

However, scuttle flies are ecologically diverse and include saprophagous as well as parasitic or omnivorous species. The larva of *M. scalaris* feeds on a broad variety of decaying organic material and adults may also land on open wounds of patients in medical facilities in order to lay eggs (wound myiasis). Potentially, pathogens can be transferred to wounds from faecal or other decaying material. The development time of the *Megaselia* species is similar to that of *D. melanogaster*.

The differentiation of fruit flies and scuttle flies may be problematic for the general public since they are similar in size and habitus.

5.6.5.8.2 Dossier requirements

Dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

5.6.5.8.2.1 Test species

A product claimed "against fruit flies" has to be tested on *D. melanogaster* and a product claimed "against scuttle flies" has to be tested on *M. scalaris.* Adult organisms should be tested, deviations should be justified and tested, i.e. if other developmental stages are claimed. For a general claim against "fruit and scuttle flies" both species, *D. melanogaster* and *M. scalaris* have to be tested. For a species-specific claim, testing against the claimed species is required.

Due to the specificity of certain active substances, e.g. pheromones, for products based on an active substance with a species-specific mode of action, only effects against fruit fly or scuttle fly species that have been tested under simulated-use and/or field conditions, depending on the type of claim, should be claimed on the product label and in the SPC.

5.6.5.8.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.8.2.2.1 Repellent products as space and/or surface treatment

For product authorisation purposes the efficacy of repellent products should be proven in:

- a simulated-use test according to the instructions for use (test design example see 5.6.5.8.2.2.1.2), or
- a field trial according to the instructions for use (test design example see 5.6.5.8.2.2.1.3).

Products applied onto surfaces may act either by evaporation or on the surface itself.

For products applied on surfaces, two porous and one non-porous surface should be used, e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete, for a general label claim as "surface treatment". The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces. For authorisation of a product to be used on a specific type of surface the efficacy for only this specific surface should be assessed.

The repellents used for the test should be identical to the product to be marketed.

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

For products intended to be used as space treatment the room size stated in the SPC and on the label must be used in the efficacy trial. For the extrapolation to larger room sizes than proven in the efficacy test a justification is necessary.

5.6.5.8.2.2.1.1 Laboratory choice test

Different set-ups are recommended for testing the repellency of adult flies and larvae. Only one appropriate set-up is required per use.

For each of those tests the following requirements have to be fulfilled:

- at least 5 replications and 5 negative controls should be used;
- environmental conditions must be specified for the test itself and during the storage of the treated surfaces/substrates or repellent product (temperature, humidity, ventilation, and photoperiod);
- for residual efficacy, flies shall be exposed to the product at several time intervals after application (including the end of the claimed period).

5.6.5.8.2.2.1.1.1 Trap Bioassay (see Appendix 18: Knaden M., et al.)

Transparent test chambers (approximately 10x8x10 cm) are equipped with a treatment trap and a control trap, each in one corner. The trap position should rotate to avoid site preference. The traps could be made from small cups or containers (30 ml) with a lid. The lids contain a hole to insert a cut micropipette tip (tip diameter 2–3 mm), that the narrower part is pointed toward inside. An attractive food source, preferably *Drosophila* fly food (see Appendix 18: Alcaine-Colet, A., et al.), is applied in both traps. Whereas the repellent is only added in one trap. 50 flies (sex ratio 1:1; 4–5 days old; not older than 7 days as aging affects the sensory abilities) that are starved for 24 hours before the experiments with water ad libitum, are transferred into the test chambers. The number of flies in and outside the traps should be counted.

5.6.5.8.2.2.1.1.2 Choice assay using the "capillary feeder" (CAFE) method (see Appendix 18: Ja, W.W., et al.)

The set-up consists of two transparent chambers, an inner chamber containing the flies and an outer chamber, filled with water. The inner chamber could be prepared by paring down a 1.5 cm diameter plastic vial to 2 cm length, with the bottom pierced to allow entry of water vapour and air from the outer chamber. Two glass micropipettes filled with a liquid medium by capillary action are inserted through the cap via truncated pipette tips. Attractive liquid food could be topped with an oil layer to minimize evaporation. The repellent is only added in one micropipette. To facilitate visualization, a red dye could be added to the medium and can be seen in the proboscis and abdomen of the fly. The number of flies feeding on both pipettes should be counted. The set-up can be conducted with flies housed individually up to groups with eight animals per chamber.

5.6.5.8.2.2.1.1.3 Double cage or glass tubes test

Experiments should be conducted in a test apparatus consisting of two cages (approximately 30x30x30 cm) connected by a tunnel. Walls should be transparent to allow better insect counting, and all surfaces should be of cleanable material, sealed and waterproof that is 100% decontaminated. Both ends of the tunnel are equipped with a gate that connects the tunnel with the cage. One cage contains the repellent whereas the opposite cage remains untreated. The application quantity of the repellent must be matched to the size of the boxes. According to the claim the floor of the cages should be of e.g. porous and non-porous substrate representing surfaces typically treated, e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete. At the start of the experiment, the repellent is applied, and 50 adult flies are released in the middle of the tunnel with both gates closed. Alternatively, a third cage could be used as a release box interconnected by two tunnels between both cages. After 10 minutes, the gates are opened, and the insects can choose the treated or control cage. The number of flies in each test cage is recorded 5, 10, 30 and 60 minutes after exposure to the repellent. The ratio of insects found in the treated vs. control cage is a measure for the efficacy of the product. Depending on the claim the test could be performed with or without food attractant.

Alternatively, the cages could be replaced by glass tubes, usually, three tubes (approximately 50 cm length each and a diameter of at least 10 cm); insects in the middle tube and two peripheral tubes, one containing the repellent; peripheral tubes should be closed by gauze to allow air circulation.

The test apparatus should be installed in a ventilated room to avoid contamination of the control cage/tube by evaporation of the repellent.

To exclude side preference effects a control treatment without active substance in any cage should be conducted with insects from the same insect population.

5.6.5.8.2.2.1.1.4 Choice bioassay for larvae (see Appendix 18: Dweck, Hany K.M., et al.)

Petri dishes (diameter: approximately 10 cm), filled with 1% agarose solution can be used as behavioural arena. The dish should be divided into two equal parts and two filter paper discs (diameter: 0.5 cm) are applied on each side of the dish at the periphery; one containing the repellent. Transfer 50 larvae (2^{nd} or 3^{rd} instar) in the centre of the Petri dish. Number of larvae on each side should be counted.

5.6.5.8.2.2.1.1.5 Choice bioassay for testing flies oviposition behaviour (see Appendix 18: Dweck, Hany K.M., et al. and Stensmyr, Marcus C., et al.)

Transparent test chambers (of approximately 50x50x50 cm) are equipped with two Petri dishes (diameter: approximately 10 cm), filled with oviposition medium, each in one corner. The repellent is only added in one Petri dish, 20 mated female flies (4–5 days old; not older than 7 days as aging affects the sensory abilities) are transferred into the test chamber. The number of eggs in each Petri dish should be counted.

5.6.5.8.2.2.1.2 Simulated-use test

Mandatory requirements:

- A choice test should be conducted with an attractive food source.
- For ambient repellents for protection of large rooms, e.g. diffusers to protect rooms with a defined volume, a double room test should be performed, with at least one 20 m^3 test room for product application. Such spatial repellent products can repel fruit and scuttle flies to protect rooms from entering and/or dispel the insects from already infested areas.
- Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.
- Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range of 20-28°C.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

For products, having a limited releasing rate into the air, e.g. the following set-up could be used. Other test designs than the following examples can be accepted if the protocol is scientifically valid.

Test design: Test rooms are equipped with an attractive food source, preferably *Drosophila* fly food (see Appendix 18: Alcaine-Colet, A., et al.), presented on a table with a height of approximately 0.5 m. Report the location of the table in the chamber. At least 200 adult free flying flies, *D. melanogaster* or *M. scalaris*, mixed sexes, are released into the room. After at least 1 hour of acclimatization the repellent product is applied according to the claim, e.g. on both porous and non-porous substrate representing surfaces typically treated e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete. The number of flies should be monitored, for at least 1 minute, at the end of the claimed efficacy period, within a defined radius, e.g. minimum 40 cm radius on the surface where the food is presented or according to the claim, around the attractive food source. For residual efficacy, flies are exposed to the product at several time intervals after application (including the end of the claimed period).

Proof of non-insecticidal efficacy: After each test run the mortality of the adult insects should be monitored.

Efficacy assessment: The potential repellent effect of the product is determined by comparing the number of flies at or around the attractive food source with the repellent (within a defined space) to the number of flies at or around the attractive control food source (negative control). Alternatively, the effect on the next generation can be used to determine the repellent efficacy.

This can be conducted after the trial by maintaining the food source in a cabin/cage free of flies in order to evaluate if some larvae hatch after 2 to 4 days. The number of larvae and/or adults found on the treated vs. control food source (negative control) is a measure for the efficacy of the product (% offspring).

5.6.5.8.2.2.1.3 Field trial

In the field trials, the product is tested in the actual use situation, for instance in an infested home, medical and veterinary facilities or warehouse and applied according to the direction for use in the SPC.

Test design: The test set-up should be similar to the one described for the simulated-use trial (see 5.6.5.8.2.2.1.2), and, for vapour-based products, reflect the claimed air volume (according to the direction for use; at least 20 m^3).

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.

Environmental conditions must be specified for the test itself and during the storage of the treated surfaces/substrates or repellent product (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range 20-28°C.

Proof of non-insecticidal efficacy, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8), has to be examined at the end of the trial.

Efficacy assessment: The potential repellent effect of the product is determined by comparing the number of flies at or around the attractive food source with the repellent (within a defined space) to the number of flies at or around the attractive control food source (negative control). Alternatively, the effect on the next generation can be used to determine the repellent efficacy. This can be conducted after the trial by maintaining the food source in a cabin/cage free of flies in order to evaluate if some larvae hatch after 2 to 4 days. The number of larvae and/or adults found on the treated vs. control food source (negative control) is a measure for the efficacy of the product (% offspring).

5.6.5.8.2.2.2 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractants in traps without PT18 active substance should be proven in:

- a simulated-use test according to the instruction for use (test design examples see 5.6.5.8.2.2.2.2), or
- a field trial according to the instruction for use (test design examples see 5.6.5.8.2.2.2.3).

The attractants, e.g. the traps, used for the test should be as similar as possible to the product to be marketed (for details see Introduction, section 5.6.5.1.3.7).

If outdoor use is claimed, the test should be performed outdoors, and the attractant product and control have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3.).

5.6.5.8.2.2.2.1 Laboratory choice test

Different set-ups are recommended for testing attractants of adult flies and larvae. A choice test similar to the tests described in section 5.6.5.8.2.2.1.1 could be used by comparing the number of flies preferring the attractant vs. attractive control food source, preferably *Drosophila* fly food.

5.6.5.8.2.2.2.2 Simulated-use test

Mandatory requirements:

- The simulated-use test should be conducted in a room of at least 20 m^3 with an attractive food source.
- Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.
- Environmental conditions must be specified for the test itself and during storage of the attractant product (temperature, humidity, photoperiod, ventilation). The temperature

would be expected to fall in the range 20-28°C. Temperature and relative humidity should be kept at a constant level (±1°C, ±10% relative humidity) throughout the test period and for all replicates.

- Traps should be tested on their own, with a negative control tested separately in an identical test setting.
- If the product is claimed to protect premises without food sources, where flies originate from near infested areas, the efficacy should be tested without food source.

Other test designs than the following examples can be accepted if the protocol is scientifically valid.

Test design: Test rooms are equipped with an attractive food source, preferably *Drosophila* fly food, presented on a table with a height of approximately 0.5 m. Report the location of the table in the chamber. At least 200 adult free flying flies, *D. melanogaster* or *M. scalaris*, mixed sexes, are released into the room. After at least 1 hour of acclimatization the attractant product/trap is applied according to the label claim. In order to prove the choice of the flies, the distance between food source and trap should be large enough to avoid accidental catching of the flies due to undirected movement. As the distance may depend on the product, the distance used in the trial should be the same as claimed on the product label and in the SPC. Number of flies is monitored at defined time intervals until the end of the claimed efficacy period, e.g. 0.5 and 1 hour after introduction of the product). For residual efficacy, flies are exposed to the product at several time intervals after application (including the end of the claimed period).

Efficacy assessment: Attraction may depends on the mode of action of pheromones. Therefore, attractants may be sex specific or sex unspecific. Attracting effectiveness of a sex unspecific product is determined by comparing the number of flies within the treated trap to the control trap. For products which affect only individuals of one sex the efficacy should be shown on the next generation. This can be conducted after the trial by maintaining the food source in a cabin/cage free of flies in order to evaluate if some larvae hatch after 2 to 4 days. The number of larvae and/or adults found on the treated vs. control food source is a measure for the efficacy of the product (% offspring).

5.6.5.8.2.2.2.3 Field trial

In the field trials, the product is tested in the actual use situation, for instance in an infested home, medical and veterinary facilities or warehouse and applied according to the direction for use in the SPC.

Test design: The test set-up should be similar to the one described for the simulated-use trial (see 5.6.5.8.2.2.2.2), and, for vapour-based products, reflect the claimed air volume (according to the direction for use; at least 20 m³).

Any bycatch of non-target insects has to be recorded and identified at least to order, preferably to the family level.

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.

Environmental conditions must be specified for the test itself and during the storage of the attractant product (temperature, humidity, photoperiod, ventilation). The temperature would be expected to fall in the range 20-28°C.

Efficacy assessment: The potential attracting effect of the product may depends on the mode of action of pheromones. Therefore, attractants may be sex specific or sex unspecific. Attracting effectiveness of a sex unspecific product is determined by comparing the number of flies within the treated trap to the control trap. For products which affect only individuals of one sex the efficacy should be shown on the next generation. This can be conducted after the trial by maintaining the food source in a cabin/cage free of flies in order to evaluate if some larvae hatch after 2 to 4 days. The number of larvae and/or adults found on the treated vs. control food source is a measure for the efficacy of the product (% offspring).

5.6.5.8.2.2.3 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.8.3 Assessment of authorisation

5.6.5.8.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for fruit flies and scuttle flies in the following way:

Products intended for use as repellents against fruit flies and scuttle flies as space and/or surface treatment:

Non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

The required results for the different trials are:

Laboratory choice-tests (not required for product authorisation):

- ≥ 80% repellency within the test period (according to the claim), from the beginning and until the end of the claimed efficacy period.

Simulated-use test:

- ≥ 80% efficacy (depending on the assessment method (see 5.6.5.8.2.2.1.2), i.e. ≤ 20% offspring or ≥ 80% repellency) within the test period (according to the claim), from the beginning and until the end of the claimed efficacy period.

Field trial:

- ≥ 80% repellency (depending on the assessment method (see 5.6.5.8.2.2.1.3), i.e. ≤ 20% offspring or ≥ 80% repellency) within the test period (according to the claim), from the beginning and until the end of the claimed efficacy period.

The product label and the SPC should state that the insect population may not be reduced to zero by the use of this type of product alone; therefore, the product should be used in combination with other products or technologies as part of integrated pest management. The label claim should be "reduces population", not "eliminates population".

Attractants without PT18 active substances

The required results for the different trials are:

- at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

Laboratory choice-tests (not required for product authorisation):

- ≥ 80% attraction compared to the negative control within the test period according to the claim, from the beginning and until the end of the claimed efficacy period.

Simulated-use test:

- ≥ 80% efficacy (depending on the assessment method (see 5.6.5.8.2.2.2.2) i.e. ≤ 20% offspring or ≥ 80% trapped test individuals) within the test period according to the claim (e.g. sex), from the beginning and until the end of the claimed efficacy period.

Field trial:

- ≥ 80% efficacy (depending on the assessment method (see 5.6.5.8.2.2.2.3) i.e. ≤ 20% offspring or ≥ 80% trapped test individuals) within the test period according to the claim, from the beginning and until the end of the claimed efficacy period.

The product label and the SPC should state that not the entire insect population might be caught by this type of product; therefore, the product should be used in combination with other products or technologies as part of integrated pest management. The label claim should be "reduces population", not "eliminates population".

5.6.5.9 Mosquitoes

5.6.5.9.1 Introduction

Mosquitoes, including different species of notably the genera *Culex*, *Aedes*, and *Anopheles*, are common pests in the EU. While mosquitoes are mainly a nuisance problem in most parts of the

EU, some species are known for their vector competence, e.g. the Asian tiger mosquito (*Aedes albopictus*) and there have been reports of local dengue transmission in France, Croatia and Spain. Chikungunya, another viral disease transmitted by mosquitoes, reached Europe in 2007 resulting in a local outbreak in northern Italy with almost 200 cases. On a global scale, mosquitoes are responsible for transmitting diseases such as malaria (*Anopheles* spp.), yellow fever (*Aedes* spp., mainly *Aedes aegypti*), dengue fever (*Ae. aegypti* and *Ae. albopictus*), Chikungunya (*Ae. aegypti* and *Ae. albopictus*), Zika (*Ae. aegypti, Ae. albopictus*) and West Nile fever (*Culex* spp.). So far most of these exotic pathogens are not endemic in Europe, but occasional outbreaks may occur, and travellers might also encounter them when visiting disease-endemic (tropical or subtropical) countries around the globe. Nevertheless, an increasing number of autochthonous infections has been reported for West Nile virus, malaria and dengue since 2012.

PT19 biocides against mosquitoes can only claim to repel or attract (in order to trap) the target organisms and to protect humans and/or animals from being bitten, but not to prevent the diseases.

5.6.5.9.1.1 Biology

Like all Diptera, mosquitoes go through four development stages. The egg, larval and pupal stages take place in stagnant water bodies such as floodplains, drainage ditches, and natural or artificial water containers. Depending on the genus, female mosquitoes lay their eggs directly on the water surface, e.g. *Culex* spp., *Anopheles* spp., or above the water line, e.g. *Aedes* spp. on damp surfaces in areas that are likely to temporarily flood. Depending on the genera, eggs are laid individually, e.g. *Aedes* spp., *Anopheles* spp., or in bundles called rafts, e.g. *Culex* spp.

Once larvae hatch, filter feeding begins near the water surface. Mosquitoes go through four larval instar stages before entering the pupal stage. The pupal stage lasts about two days, during this time mosquitoes do not feed. Once completed, the adult emerges and enters the terrestrial environment. Mating begins a few hours to days after emergence. Once mated, the female starts to search for a blood meal. Humans, birds, and other animals are potential blood hosts, with some mosquito species preferring human blood to other animals.

The adult female mosquito locates her host through visual cues, heat, moisture, and most importantly host-derived odour plumes that represent a strong olfactory cue (exhaled carbon dioxide and skin emanations). Once located, the host-seeking female mosquito will attempt to bite and eventually take up a blood meal. This blood meal is partially digested and used for the development of eggs. About three to four days after the blood meal, the female will lay 50 to 500 eggs. Most species lay eggs more than once during their life-span, thus, shortly after oviposition females will start to seek their next blood meal. This particular behaviour turns some mosquito species into very effective vectors of disease. If they feed on an infected host, they can become infectious themselves, provided that they are capable vectors of the pathogen, and transmit the pathogen to their next host.

5.6.5.9.2 Dossier requirements

General dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

In order to eliminate the risk of disease transmission to human volunteers in field settings, field trials with repellents against mosquitoes are not required for authorisation of products applied on humans or clothing (for details see Introduction, section 5.6.5.1.3.4.3). In the case of products applied to domestic animals, field trials can be less stressful for the animals and should therefore be accepted instead of simulated-use test.

For authorisation of products applied on human or animal skin efficacy must be proven with the recommended label dose and according to the claimed mode of application given on the label and in the SPC. The dose used in the efficacy studies should be covered by human health risk assessment.

5.6.5.9.2.1 Test species

Products intended for use as repellent for human or animal skin, clothing:

For a general claim against mosquitoes, repellent efficacy testing must be conducted with at least one mosquito species from each of the three genera *Culex, Aedes* and *Anopheles*. *Culex* spp. is the most common mosquito in Europe. *Aedes* spp. are the most aggressive mosquitoes.

For a species-specific label claim, testing against the claimed species is required.

When use against tropical mosquitoes or use in tropical areas is claimed, it should be specified against which mosquito spp. the product is effective and these should be tested. The minimal efficacy should be proven against three genera: *Aedes* spp., *Culex* spp. and *Anopheles* spp. The choice of test species should be justified.

Products intended for use as general surface and spatial repellents:

For a general claim against mosquitoes, repellent efficacy testing must be conducted with at least one mosquito species from each of the two genera *Culex* and *Aedes*. *Culex* spp. is the most common mosquito in Europe. *Aedes* spp. are the most aggressive mosquitoes.

For a species-specific label claim, testing against the claimed species is required.

When use against tropical mosquitoes or use in tropical areas is claimed, repellent efficacy testing must be conducted with at least one mosquito species from each of the three genera *Culex, Aedes* and *Anopheles*.

General requirements for all products against mosquitoes:

Due to the specificity of certain active substances, e.g. pheromones, for products based on an active substance with a species-specific mode of action, only effects against mosquito species that have been tested under simulated-use and/or field conditions, depending on the type of claim, should be claimed on the product label and in the SPC.

For insects from laboratory rearing used in the efficacy studies the test species, strain and age should be reported (for more details see Introduction, section 5.6.5.1.3). All test mosquitoes should be non-blood fed, but not starved. Sugared water has to be provided before and during the test in order to keep the mosquitoes fit for testing.

5.6.5.9.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.9.2.2.1 Products intended for use as topical repellents for human skin

For product authorisation purposes the efficacy of repellent products targeting the use on human skin should be proven in a simulated-use test according to the instructions for use (test design example see 5.6.5.9.2.2.1.2).

The repellents used for the test should be identical to the product to be marketed.

CPT values must be determined in a simulated-use test. As field studies are not evaluated as key studies, data from the field studies cannot be used to determine CPT values.

5.6.5.9.2.2.1.1 Laboratory test

Different set-ups are recommended for testing repellent products targeting the use on human skin. Only one appropriate set-up is required per use.

For each of those tests the following requirements have to be fulfilled:

- at least 10 replications and 10 negative controls should be used;
- environmental conditions must be specified for the test itself (temperature, humidity, ventilation, and photoperiod). The temperature would be expected to fall in the range between 19-29°C.

5.6.5.9.2.2.1.1.1 Olfactometer test

Y-tube olfactometer tests can be performed to measure the behavioural responses of host-seeking female mosquitoes towards volatile repellent active ingredients in the presence of natural host odours, see Appendix 18: Geier M. and Boeckh J.. Each olfactometer consists of a transparent acrylic glass base leg, followed by a decision chamber and two branches which terminate into Teflon chambers, where the test stimuli are introduced. The system is supplied with a constant purified air stream heated up to 26±1°C and humidified to a relative humidity

of 70±5%. Rotating doors in both branches, as well as at the downwind end of the base leg, allow the release and entrapment of the test mosquitoes. Cohorts of 15-20 host seeking female mosquitoes are attached to the apparatus at its downwind end.

Repellent test formulations are applied on filter paper strips (approximately 1 cm×3 cm) which can be suspended into one of the Teflon chambers. A forefinger or a hand is then inserted into the Teflon chamber behind the paper strip and the rotating door of the base leg is opened. Mosquitoes are allowed to fly upwind for 30 seconds and decide between the test branch with volatile stimuli and the control branch with a finger or a hand without repellent. At the end of a test, the rotating doors are closed and the number of mosquitoes that migrated from the release cage (= active), the number of mosquitoes inside the test cage (where the stimuli were applied), and the number of mosquitoes in control cage (with filtered air) are documented. At the conclusion of a test, the airflow in the apparatus is inverted and mosquitoes are lured back into the release cage by the palm of the hand and the next of four y-tubes can be used for testing. Treatments are tested in randomized order, and after each run, the control branch and test branch are changed to avoid position or adaptation effects. Each single compound or mix are tested in 10 replicates. All treatments are tested against a control of the forefinger and a paper strip treated with solvent only.

5.6.5.9.2.2.1.1.2 Blood Feeding Assays (see Appendix 18: Mulatier M., et al.)

The set-up consists of a container (approximately 10 cm length and 7 cm diameter) containing 25 female mosquitoes and covered with a piece of polyester net previously impregnated with the repellent. Containers are placed on a membrane allowing the mosquitoes to feed through it. After 1 h of exposure, the proportion of blood-fed females can be counted.

5.6.5.9.2.2.1.2 Simulated-use test

In this test, the repellent is applied to the skin of the forearm of a human volunteer and regularly exposed to caged populations of host-seeking pathogen-free mosquitoes. Different set-ups can be used: "arm-in-cage", "arm-to-cage" or a room test.

In all set-ups a minimum landing rate on the untreated skin must be achieved. Due to the different aggressiveness of the mosquito species the following landing rates, corresponding to a worst-case landing pressure in the field (see Appendix 18: Moreno-Gómez, M., Bueno-Marí R., Drago A., et al.), must be achieved:

- *Aedes* spp. 20 landings/minute;
- *Culex* spp. 5 landings/minute;
- *Anopheles* spp. 5 landings/minute.

The density and number of animals required to achieve the required landing rate may vary between mosquito species and between different studies.

Environmental conditions must be specified for the test itself (temperature, humidity, light intensity). The same parameters should be chosen as test conditions for tropical and non-tropical regions: temperature 27°C±2°C and relative humidity of 75%±5%.

5.6.5.9.2.2.1.2.1 Volunteers

Efficacy data of at least 10 different volunteers, volunteers with different hairiness of the arms, different genders; age: 18-65 years, should be collected since repellence/attractiveness to mosquitoes varies considerably between human individuals. For CPT calculation valid data of at least 10 different volunteers must be used. Volunteers' attractiveness is measured by exposing the untreated skin to caged populations (population density, cage size see below) of host-seeking, pathogen-free female mosquitoes, and the results must be presented in the report.

To avoid unnecessary blood feeding, the volunteers are allowed to softly shake off the mosquitoes after landing. In case biting activity is lower, a new cage with fresh mosquitoes should be used or additional mosquitoes could be added and reported.

Volunteers need to be fully informed about the aim, the procedure, and the expected duration of a study. The study should be carried out in compliance with the national ethics regulation[55].

[55] Declaration of Helsinki; https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects

They also need to be informed about potential side effects such as allergic skin reactions caused by the product. Their participation is voluntary and can be recalled at any time before and during the study. 12 hours before and during testing, volunteers should avoid nicotine, alcohol, fragrance (perfumes, body lotions, soap, etc.) and repellent products. Any side effect observed during the test should be mentioned in the test report.

Prior to the application of the repellent, the skin is washed with fragrance-free soap and rinsed with water. The hand should be covered with a glove that mosquitoes cannot bite through during each exposure to the test insects. If the entire forearm is to be treated with repellent, the skin area should be estimated according to recommendations of the WHO (2009): the mean circumference is calculated by measuring the circumference at the wrist and elbow and multiplied with the length of the arm (between wrist and elbow).

Alternatively, repellents can be applied to a defined area on the forearm, e.g. a 100 cm^2 window. The rest of the arm will then be protected from bites by a custom-made sleeve (flexible material that prevents biting, edges are not treated with the product; contamination should be prevented). The use of a defined area adds to standardisation of test conditions; therefore, the use of a sleeve is recommended. Repellent products have to be applied at the recommended label dose.

5.6.5.9.2.2.1.2.2 Arm-in-Cage test

A simulated-use test with the following criteria could be conducted to determine realistic CPT values.

Test cage:

- a volume between 27 L and 64 L can be used. The volume should be at least 27 L to have enough space in the cage for the forearm;
- both a rectangular and a square shape are suitable;
- the front side of the cage contains a sleeve for the introduction of the forearm and as a barrier for the mosquitoes to escape.

Mosquito density:

- the density and number of animals required to achieve the required landing rate may vary between different trials. However, the following information may serve as a guide: A density between 1 female/840 cm^3 and 1 female/640 cm^3 should be chosen, e.g. 32-42 females in 27 L; 75-100 females in 64 L. The number of mosquitoes can vary according to the mosquito species.

Exposure period:

- the treated skin should be exposed for 3 min in regular intervals.

In each exposure period, prior to the efficacy test with the treated arm, the landing pressure of the pathogen-free test mosquitoes needs to be verified with the other, untreated arm.

5.6.5.9.2.2.1.2.3 Arm-to-Cage test (see Appendix 18: Obermayr, U., et al.)

Test cages are usually rectangular and have a volume of 27 L, e.g. 41x41x16 cm. However, slightly larger cages can also be used, as long as the minimum mosquito landing pressure of 20 landings per minute is met. The floor of the cage contains a test window, e.g. 60 cm^2 exposed skin area in a 27 L volume cage for the exposure of the skin, in contrast to the arm-in-cage test the arm is not exposed inside the test cage. Mosquito landing pressure and exposure period should follow recommendations made in section 5.6.5.9.2.2.1.2.2 "Arm-in-Cage Test". The treated skin area on the forearm is marked and should be larger than the test window in the floor of the cage to ensure that the exposed skin is entirely treated with repellent. Prior to the efficacy test of the treated arm, the landing pressure of the pathogen-free test mosquitoes needs to be verified with the other, untreated arm.

5.6.5.9.2.2.1.2.4 Room test (see Appendix 18: Moreno-Gómez, M., Bueno-Marí R., Bowman, G.R., et al.)

Tests should be conducted in free-flight, mosquito-proof rooms with a volume of at least 25 m^3. For day active mosquitoes the room should be illuminated by external lighting, e.g. 150 W halogen fluorescent lighting, on the ceiling to eliminate shadows and white walls allow a better insect counting. All surfaces should be of a cleanable material, sealed and waterproof to avoid the accumulation of the product in the room.

A sufficient number of female mosquitoes pre-selected for host-seeking behaviour are used per trial to achieve the required landing rate on the untreated arm. Test mosquitoes are allowed to acclimatize for 15 minutes in the test room before the volunteer enters the room. The landing rate should be measured on the untreated arm of the volunteer at each test interval. In case this minimum landing pressure is not achieved, additional mosquitoes can be added. Over the entire test an average of the required landing rate must be achieved. Due to the natural behaviour, variations in mosquito activity during the day are likely. Therefore, landing rates below and above the required landing rate at the different test intervals are acceptable as long as the mean landing rate remains above the required landing rate during the whole observation period.

Volunteers: Criteria described in section 5.6.5.9.2.2.1.2.1 must be fulfilled. During the test, a light beekeeper suit, gloves and white hospital booties should be used to protect the volunteer from getting bitten. The untreated arm is covered with the suit, whereas the treated arm should be protected from bites and abrasion of the product by a "tube" of flexible material, see Figure 14; edges are not treated with the product; contamination should be prevented. A handle inside the tube allows the volunteer to hold it. The tube is only used when the volunteer is in the cabin, the rest of the day the arm is exposed to the air. The volunteer enters the cabin with both forearms covered and walks through the cabin (approximately 2 minutes) until discovered by the mosquitoes. Then the volunteer stops walking, exposes the untreated arm and counts the number of landings, e.g. for *Aedes albopictus* for an exposure period of 3 minutes. The exposure period should be the same during the study and should be adapted to the mosquito species. After the landing rate has been determined as sufficient, the volunteer covers the untreated arm and immediately exposes the treated arm. The exposure period starts once the treated arm is fully exposed.

Figure 14: Room test volunteer wearing a light beekeeper suit, gloves and white hospital booties and one arm is protected by a "tube" of flexible material

After each efficacy test, the room needs to be cleaned thoroughly before the next test is initiated.

5.6.5.9.2.2.1.2.5 Efficacy assessment

Mosquito strain, age and larval diet should be recorded.

While testing for repellence, an endpoint for failure of repellence for subjects treated with the recommended label dose should be selected. Efficacy failure in a test to determine CPT is the time from application of a repellent until efficacy failure by a confirmed event (definition see Introduction, section 5.6.5.1.5). Repellent efficacy should be based on the median or mean CPT (CPT calculation see Introduction, section 5.6.5.1.5).

The claimed CPT for a general claim should correspond to the shortest CPT (mean or median) among all tested species and should be stated on the label and SPC. For specific claims the mean or median for each species respectively may be stated separately on the label and SPC.

Proof of non-insecticidal efficacy, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8): After the test or in a separate test, test mosquitoes are provided with a 10% sugar solution and maintained within the test cage or test room to observe whether insecticidal effects could be caused by contact/exposure to the test repellent product. The mortality of the insects should be monitored at the end of the test.

5.6.5.9.2.2.1.3 Field trial

In order to eliminate the risk of disease transmission to human volunteers in field settings, field trials are not required for authorisation of products applied on human skin. Pre-existing studies may be submitted as additional information because field trials are not assessed as key studies.

If field trials are conducted, they must take place in an area with an appropriate mosquito biting pressure and at a time when the relevant mosquito species are active. The required biting pressure will depend on the species:

- *Aedes* spp.: at least 1 probe or bite/min is required,
- *Anopheles* spp.: at least 1 probe or bite/min is required,
- *Culex* spp. can be less active. The biting pressure chosen for test conduction should be justified in the test report.

These tests should preferably take place in Europe or other relevant regions according to the claims, e.g. tropical regions. Where tests in Europe are not possible the conditions and mosquito species must be confirmed, and their relevance justified.

Field trials should follow the WHO or EPA protocol, see Appendix 18. In-house protocols are also acceptable; any deviations should be justified.

Field trials allow the evaluation of topical repellents against wild mosquito populations under realistic conditions at the recommended label dose. In contrast to simulated-use tests, they are, however, subjected to greater variation regarding biting activity, mosquito species, population density, temperature, humidity, light conditions, etc. Since these factors cannot be controlled, field trials should be conducted in a field site in an environmentally distinctive habitat, e.g. forest, wetland, where the predominant mosquito species differ.

Volunteers: Criteria described in section 5.6.5.9.2.2.1.2.1 must be fulfilled. Volunteers trained on the use of an aspirator can serve as their own control on the other, untreated, arm or leg. Alternatively, an equal number of untreated volunteers could be included in the field trial, as a negative control.

The forearm (between wrist and elbow) or lower leg (between knee and ankle) serves as the treatment area. The skin area should be measured and treated as described in section 5.6.5.9.2.2.1.2.1. Product application must follow the claimed instruction of use. The treated skin will be exposed in regular intervals for not less than 3 minutes. The frequency of assessments will be not less than every hour until CPT ends (occurrence of the first confirmed bite, see Introduction, section 5.6.5.1.5). Exposure periods should be synchronized with the time when the relevant species are abundant. The treatment of arms or legs should also be consistent with the feeding preference of the target species. Time of day at which volunteers are treated and at which exposure started and ended should be reported along with the weather conditions (temperature, humidity, precipitation, wind speed, light intensity, cloudiness). Mosquitoes that probe or bite during an efficacy test can be collected, where possible, for later identification. Before the study is conducted, the presence of the relevant mosquito species has to be verified. This should be documented in the study report.

5.6.5.9.2.2.2 Repellents applied on clothing both for humans or animals

For product authorisation purposes the efficacy of repellent products applied on clothing for humans should be proven in a simulated-use test according to the instructions for use (description see 5.6.5.9.2.2.2.1)

CPT values must be determined in a simulated-use test. As field studies are not assessed as key studies, data from the field studies cannot be used to determine CPT values (definition and calculation see Introduction 5.6.5.1.5).

In order to eliminate the risk of disease transmission to human volunteers in field settings, field trials with repellents against mosquitoes are not required for authorisation of products applied on clothing to protect human skin (for details see Introduction, section 5.6.5.1.3.3.3). If field trials are conducted, they must take place in an area with an appropriate mosquito biting pressure (see 5.6.5.9.2.2.1.3) and at a time when the relevant mosquito species are abundant. These tests should preferably take place in Europe or other relevant regions according to the claims (e.g. tropical regions). Where tests in Europe are not possible the conditions and mosquito species must be confirmed, and their relevance justified.

For product authorisation purposes the efficacy of repellent products applied on clothing for animals should be proven in:

- a simulated-use test according to the instructions for use (description see 5.6.5.9.2.2.3.2), or
- a field trial according to the instructions for use.

The repellents used for the test should be identical to the product to be marketed.

Proposed label claims regarding the protection of a specific type of fabric must be simulated, i.e. the same type of fabric has to be used in the simulate-use test.

Proposed label claims regarding the performance of the product must be simulated in the study (for details see Introduction, section 5.6.5.1.3.5).

5.6.5.9.2.2.2.1 Simulated-use test

The efficacy of products intended for use on clothes or fabrics for humans must be proven in a similar test as described in section 5.6.5.9.2.2.1.2 by covering the test arm with a treated cloth and the control arm with untreated cloth, or simulating the normal use of the treated fabric in case of products to protect animals (see 5.6.5.9.2.2.3.2), depending on the claim. The fabric used has to be representative of the fabrics commercially used, i.e. thick uniform cotton if the field of use is military clothing, etc.

Proof of non-insecticidal efficacy, if it cannot be waived (for details see General Introduction, chapter 5.6.5.1.3.8): After the test, test mosquitoes are provided with a 10% sugar solution and maintained within the test cage or test room to observe whether insecticidal effects could be caused by contact/exposure to the test repellent product. The mortality of the insects should be monitored at the end of the test.

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation).

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for detailed information regarding the requirements see General Introduction, chapter 5.6.5.1.4.1.3).

The efficacy data should be relevant to prove the submitted claims. Therefore, the efficacy of biocidal products that are limited to a body area e.g. collars for animals must be proven for the whole test individual that should be protected. If only specific body areas intend to be protected, e.g. ears, udders, etc. then the efficacy for the claimed area must be proven.

For specific claims (prevention of bites through/prevention of bites next to the treated clothes), relevant tests have to be submitted.

5.6.5.9.2.2.3 Products intended for use as topical repellent for animals

For product authorisation purposes the efficacy of repellent products applied on animals should be proven in:

- a simulated-use test according to the instructions for use (test design example see 5.6.5.9.2.2.3.2), or
- a field trial (test design example see 5.6.5.9.2.2.3.3).

Topical repellents for animals should be tested against the targeted mosquito species and on the animal, e.g. dog, horse, etc., that shall be protected. Dogs should be used as a substitute for cats, as testing with cats can imply certain animal welfare issues.

Proposed claims regarding the performance of the product must be simulated in the study. For the claim "unaffected by washing", the label and the SPC must indicate how often the animal can be washed without reducing the efficacy of the biocidal product. In the efficacy test, the test individuals should be repeatedly washed with a non-insecticidal, fragrance-free shampoo during the trial according to the number of wash cycles indicated.

The repellents used for the test should be identical to the product to be marketed.

Test animals: It is recommended to include at least 10 animals per treatment/control group of different breed and sex, i.e. for testing on entire animals at least 10 treated and 10 control individuals are necessary since repellence/attractiveness to mosquitoes varies considerably between animal individuals.

Depending on the animal species, several factors e.g. hair length, the thickness of the coat, self-grooming, etc. might impact the efficacy. These factors should be taken into account in the demonstration of the efficacy. The two groups should be kept separately, to avoid contact of the control animals with the treated fur.

The product should be applied according to the claim. Claimed application rates should take into account the type and weight of the animals.

It is necessary that animals come from suitable, e.g. non-smoking households. Every side effect observed during the test should be mentioned in the test report. 24 hours before and

during testing, animals should not be treated with fragrance (perfumes, soap, etc.). The test animal should not be protected by any previous repellent and/or insecticidal residuals caused by previous treatment. Concerning endo-antiparasitic agents this is acceptable, but details (product, dose, application rate, application method, date of the last treatment, and residual efficacy) must be given in the report and the controls and the treated groups must have the same status. A safety margin for externally applied anthelmintics is 4 weeks after the end of the last application. The history of the treated and the control groups should be comparable. The status of the animals should be specified and only animals for which an insecticidal effect can be excluded should be included in the study. Due to feasibility and cost-effectiveness, it would be possible to use 10 animals one week (5 each in the treatment and control groups) and then use the same individuals again in the second week, but swap groups. Each animal would then act as its own control. However, any residual activity of the product has to be excluded.

The investigator is reminded that the validity of the results is directly related to the degree of variability in the test. Increasing the number of test animals could increase the reliability of the test results.

5.6.5.9.2.2.3.1 Laboratory test

A choice test similar to the tests described in section 5.6.5.9.2.2.1.1 could be used.

5.6.5.9.2.2.3.2 Simulated-use test

In the simulated-use tests, the product is applied according to the instructions for use. If the product is acting at very close range it may be possible to make the evaluation by treating parts of the host, like one treated side of the neck compared with the equivalent area on the other, untreated, side of the neck. The advantage of such a setup is the neutralisation of individual host differences in mosquito-attraction. The control body part should resemble the treated body part, e.g. front-half vs back-half or left side vs right side of the animal. In case of a restricted application, e.g. collar, a spot-on product with a claim of entire body protection or for products acting in a wider range over the entire body the test should permit to validate the efficacy of this kind of application by testing the entire animal. In this case, untreated animals must be used as a control.

The detailed measurements will depend on the size of the animal, and the following dimensions adapted from the simulated-use test on humans should be used only as guidance.

Test animals: The attractiveness of the animal should be validated before the trial. Animal's attractiveness can be measured by exposing the untreated skin to caged populations of host-seeking female mosquitoes. Repellent products have to be applied at the recommended dose and according to the claimed mode of application. Claimed application rates should take into account the type and weight of the animals. The animals tested should be representative for the claims.

The parameter for determining the number of mosquitoes in the cages is the landing rate. For details see 5.6.5.9.2.2.1.2.

Mosquitoes are attached to the treated skin in screened cages to force them on the defined treated area and landing and biting behaviour is documented in regular intervals, see Appendix 18: EPA and EMA Guidelines.

Alternatively, test animals could be placed inside a screened cage with host-seeking female mosquitoes. Untreated body parts should be covered to prevent excessive biting.

Environmental conditions must be specified for the test itself (temperature, humidity, light intensity). The temperature would be expected to fall in the range of 27°C±2°C and relative humidity of 75%±5%.

Proof of non-insecticidal efficacy, if it cannot be waived (for detail see Introduction, section 5.6.5.1.3.8): After the trial, test mosquitoes are provided with a 10% sugar solution and maintained within the test cage to observe whether insecticidal effects could be caused by contact/exposure to the test repellent product. The mortality of the insects should be monitored at the end of the test.

Efficacy assessment should be based on CPT, definition and calculation, see Introduction, section 5.6.5.1.5.

The claimed CPT for a general claim should correspond to the shortest CPT (median or mean) among all tested species and should be stated on the label and SPC. For specific claims, the mean or median for each species respectively may be stated separately on the label and SPC.

Authorisation of products applied with a dose independent of animal size can be granted up to the bodyweight of the heaviest test animal.

5.6.5.9.2.2.3.3 Field trial

For PT19 products which are applied on animals, no common protocols are currently available. However, several guidelines for the evaluation of ectoparasiticides are available, see Appendix 18. These guidelines can be adapted for the demonstration of the efficacy of repellent products to be applied on animals.

5.6.5.9.2.2.4 Products intended for use as general surface treatment

The efficacy of general surface treatment products should be proven either in:

- a simulated-use test according to the instructions for use, or
- a field trial according to the instructions for use and additionally a laboratory trial testing the required different surfaces.

Products applied onto surfaces may act either by evaporation or on the surface itself.

For products applied on surfaces, two porous and one non-porous surface should be used, e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete, for a general label claim as "surface treatment". The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces. For authorisation of a product to be used on a specific type of surface the efficacy for only this specific surface should be assessed.

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.

Environmental conditions must be specified for the test itself, and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation).

For residual efficacy, mosquitoes are exposed to the product at several time intervals after application (including the end of the claimed period).

Proposed label claims regarding the performance of the product should be simulated in the study. For example, for the claim "unaffected by cleaning/vacuuming", the surface should be repeatedly cleaned during the trial (for details see Introduction, section 5.6.5.1.3).

The repellents used for the test should be identical to the product to be marketed.

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

The repellents used for the test should be identical to the product to be marketed.

Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

5.6.5.9.2.2.5 Products intended for use as spatial repellents

For product authorisation purposes the efficacy of spatial repellent products, including repellent emanators (electronic vaporizers, candles, wristbands, passive vaporizers for volatile pyrethroids, coils) should be proven in:

- a simulated-use test according to the instructions for use (test design example see 5.6.5.9.2.2.5.1), or
- a field trial according to the instructions for use (test design example see 5.6.5.9.2.2.5.2).

When the label claim says that the product should be used in ventilated rooms the opening of windows and doors should be simulated in the test.

The room size stated in the SPC and on the label must be used in the efficacy trial. For the extrapolation to larger room sizes than proven in the efficacy test a justification is necessary.

If a claim states that the product prevents insects from landing/probing, a simulated-use test proving the landing/probing inhibition with a volunteer in the room is necessary.

If a claim states that the product prevents insects from entering a space, a simulated-use test proving the entry reduction is necessary.

In case of a specific claim regarding the use in specific climate conditions, e.g. high temperature or tropical conditions, the efficacy must be proven under these conditions.

Environmental conditions must be specified for the test itself, and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation).

If outdoor use is claimed, the test should be performed outdoor and the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

The repellents used for the test should be identical to the product to be marketed.

5.6.5.9.2.2.5.1 Simulated-use tests

Conducting simulated-use tests ensures that the mosquitoes are pathogen-free, that a known number of mosquitoes of a known age is used and that the distance between the point at which the mosquitoes are released and the source of the chemical stimulus can be defined, which allows the estimation of the protective area (especially important in outdoor evaluation). Other test designs than the following examples adapted from WHO (5.6.5.9.2.2.5.1.1 Landing/probing inhibition test; 5.6.5.9.2.2.5.1.2 Reduction of entry into an area) can be accepted if the protocol is scientifically valid.

Mandatory requirements:

- Replicates: A minimum of 5 independent replicates with different volunteers (each treatment and negative control) should be performed.
- A sufficient number of female mosquitoes pre-selected for host-seeking behaviour are used per trial to achieve a minimum landing rate. The landing rate is determined after the host is discovered by the mosquitoes (approximately after 2 minutes). The density and number of mosquitoes required to achieve this rate may vary between different trials. Over the entire test an average of the required landing rate must be achieved. Due to the natural behaviour, variations in mosquito activity during the day are likely. Therefore, landing rates below and above the required landing rate at the different test intervals are acceptable as long as the mean landing rate remains above the required landing rate during the whole observation period.
- Required minimum landing rates: Due to the different aggressiveness of the mosquito species the following landing rates, corresponding to a worst-case landing pressure in the field, must be achieved:
  - *Aedes* spp. 20 landings/minute;
  - *Culex* spp. 1 landing/minute;
  - *Anopheles* spp. 1 landing/minute.
- For residual efficacy, several trials should be conducted, in which the mosquitoes are exposed to the product at several time intervals after application (including the end of the claimed period).
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

5.6.5.9.2.2.5.1.1 Landing/probing inhibition test

The following design example for a simulated-use test was adapted from WHO, see Appendix 18. Other test designs than the following examples can be accepted if the protocol is scientifically valid.

Products that are intended to be used indoors:

Tests should be conducted in free-flight rooms (mosquito-proof and well ventilated by opening the door and/or window for a minimum of 1 air exchange/hour, see Appendix 18: te Biesebeek, J.D., et al.) that measure at least 30 m^3. The test product is installed and activated according to the label claim. Test mosquitoes are allowed to acclimatize for 1 hour under conditions similar to the test room and then released into the test room. The trained volunteer or a trained investigator documents landing and probing behaviour. After each efficacy trial, the room needs to be cleaned thoroughly before the next trial is initiated. The efficacy of the repellent product is compared to control tests without product or with the pre-treatment.

Products that are intended to be used outdoors:

Tests can be performed in experimental open areas of a minimum of 30 m^3 inside screened enclosures. Ideally, several areas should be available to allow simultaneous comparisons. The product is installed according to the label claim and a volunteer is positioned at the centre of the test area to perform human landing collections. Female mosquitoes are released outside of the test area. Landing and probing behaviour is documented for defined periods of time that depend on the product label specifications Tests should be conducted during the main biting activity of the tested species. Temperature, humidity, wind speed and direction should be recorded for the duration of each replicate.

Efficacy assessment: Percent landing/probing inhibition is based on the number of mosquitoes landing/probing in the control subtracting from this the number of mosquitoes landing/probing in the treated room and divided by the number of mosquitoes landing/probing in the control.

Proof of non-insecticidal efficacy: In the test chamber a cage (most appropriate consisting of mosquito net ensuring a good ventilation) containing mosquitoes provided with a 10% sugar solution is maintained for 1 hour during the test to observe whether insecticidal effects could be caused by exposure to the test repellent product. Mortality of the insects should be monitored at the end of the test.

5.6.5.9.2.2.5.1.2 Reduction of entry into an area

A spatial repellent is applied to protect an area of X m^2.

The following design example for a simulated-use test was adapted from WHO, see Appendix 18. Other test designs than the following examples can be accepted if the protocol is scientifically valid.

Products that are intended to be used indoors:

Tests are conducted in two adjacent rooms that allow mosquito movement from one room to the other, e.g. through a window opening. The test product is installed according to the label claim in one room, while mosquitoes are released in the other. The number of entering mosquitoes is documented for 10-15 minutes every hour by one trained volunteer or a trained investigator until the end of the claimed period. Between these observation periods, the connecting window should be kept closed, in order to avoid accumulation of the product in both rooms and to maintain a gradient of the active substance.

Products that are intended to be used outdoors:

Test should be performed in outdoor screened cages separated into two compartments that allow mosquito movement from one room to the other. The test product is installed according to the label claim in one compartment, while mosquitoes are released in the other. A volunteer sits down in the compartment without the mosquitoes at a set distance to the treatment device or control, according to the label claim, if applicable. The number of entering mosquitoes is documented for certain observation periods, e.g. at hour 1, hour 2 and hour 3. Tests should be conducted during the main biting activity of the tested species. The number of entering mosquitoes is documented for defined periods of time that depend on the product label specifications. Temperature, humidity, wind speed and direction should be recorded for the duration of each replicate.

Efficacy assessment: Percent reduction of entry is based on the number of mosquitoes entering the test area in the control subtracting from this the number of mosquitoes entering the treated test area/room and divided by the number of mosquitoes entering the test area in the control.

Proof of non-insecticidal efficacy: In the test chamber a cage (most appropriate consisting of mosquito net ensuring a good ventilation) containing mosquitoes provided with a 10% sugar solution is maintained for 1 hour during the test to observe whether insecticidal effects could be caused by exposure to the test repellent product. Mortality of the adult insects should be monitored at the end of the test.

5.6.5.9.2.2.5.2 Field trial

The repellent effect should be demonstrated for each mosquito species claimed.

The product should be applied according to the claim.

For indoor evaluations, several houses should be used, when feasible. Houses should be well described, especially with regard to the conditions relevant to product efficacy, including estimates of indoor volume and air ventilation, e.g. sealed or gapped walls, number of windows, doors, or eave area. Houses are randomized to receive either active (formulated spatial repellent) or control (placebo; inert ingredients alone) treatment during the trial. Collectors should be 'blinded' to treatment allocation.

In outdoor evaluations, spatial repellent product or control should be allocated randomly to comparable outdoor spaces with human exposure.

Replicates: A minimum of 3 independent replicates, i.e. 3 different houses, (each treatment and negative control) should be performed.

Efficacy assessment: Percent landing/probing inhibition or reduction of entry is based on the number of mosquitoes landing/probing/entering in the control subtracting from this the number of mosquitoes landing/probing/entering in the treated room and divided by the number of mosquitoes landing/probing/entering in the control.

5.6.5.9.2.2.6 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractants in traps without an active substance according to PT18 should be proven in:

- a simulated-use test according to the instruction for use (test design example see 5.6.5.9.2.2.6.2), or
- a field trial according to the instruction for use (test design example see 5.6.5.9.2.2.6.3).

Environmental conditions must be specified for the test itself and during storage of the attractant product (temperature, humidity, photoperiod, ventilation).

If outdoor use is claimed, the test should be performed outdoors and the attractant product and control have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

The attractants, e.g. the traps, used for the test should be as similar as possible to the product to be marketed (for details see Introduction, section 5.6.5.1.3.7).

5.6.5.9.2.2.6.1 Laboratory test

An olfactometer test similar to design described under 5.6.5.9.2.2.1.1.1 can be used.

5.6.5.9.2.2.6.2 Simulated-use test

As no test guidelines are currently available, any inhouse protocol may be used, if the protocol is scientifically valid. A test set-up similar to the ones described above (5.6.5.9.2.2.5.1) should be used where suitable. Conditions should mimic the claimed use.

Replicates: Tests should be performed with at least 5 replicates, and 5 negative controls should be used.

The number of mosquitoes released into the room or enclosure needs to be recorded and put into relation to the captured mosquitoes.

5.6.5.9.2.2.6.3 Field trial

In the field trials, mosquito attractants that are intended to be used with trapping systems or within other "lure and kill" products should be evaluated against the claimed target species. Attractants should be tested in combination with a system that allows the documentation of

captured specimen, e.g. a commercial mosquito trapping device (see Appendix 18: Vythilingam, I. et al., Kline, D.L., et al., Okumu, F., et al.). The location of the attractant within the trap or system needs to be documented.

Any attractant should be tested in a comparison between a trapping device equipped with the attractant to an untreated reference device of the same model, in a 2x2 Latin square design. The attractant must show a statistically significant increase in catch rates, as compared to the control trap.

Field trials need to be conducted in a biotope with a natural occurrence of the target mosquito species.

The distance between test locations depends on the claim, on the species and the environmental conditions (suggested distance: 30-50 m). Test locations should be far enough apart to ensure that interactions between traps (attractants) do not occur, but near enough to experience similar weather, biotope and comparable mosquito densities. Traps are installed according to the manufacturer's recommendations. Trap operation hours depend on the target species main activity: all traps should be switched on at the same time and be operated during peak activity hours of the target species, e.g. between dusk and dawn. Alternatively, traps can be operated for 24 hours. Operation hours need to be the same for all treatments during the entire experiment. At the end of a catch period, mosquito collections are removed from the trap and stored for later counting and identification.

Any bycatch of non-target insects has to be recorded and identified at least to order, preferably to the family level.

5.6.5.9.2.2.7 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.9.3 Assessment of authorisation

5.6.5.9.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for mosquitoes in the following way:

Products intended for use as repellent for human or animal skin, clothing:

Non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

The required results for the different trials are:

Laboratory tests (not required for product authorisation):

- 100% repellency for the claimed time period.

Simulated-use tests and field trials:

- during the claimed protection period complete protection should be proven expressed as mean or median CPT;
- CPT should correspond to the shortest one among the tested species; for specific claims the mean or median for each species can be stated on the label).

Products intended for use as general surface and spatial repellents:

Non-insecticidal efficacy has to be proven in a simulated-use test (for details see Introduction, section 5.6.5.1.3.8).

The required results for the different trials are:

Laboratory tests (not required for product authorisation):

- 95% repellency for the claimed time period.

Simulated-use tests:

- ≥ 80% percent landing/probing inhibition within the test period according to the claim, from the beginning and until the end of the claimed efficacy period, or

- ≥ 80% reduction of entry within the test period according to the claim, from the beginning and until the end of the claimed efficacy period.

Field trials:

- ≥ 80% percent landing/probing inhibition within the test period according to the claim, from the beginning and until the end of the claimed efficacy period, or
- ≥ 80% reduction of entry within the test period according to the claim, from the beginning and until the end of the claimed efficacy period.

Attractants without PT18 active substances:

The required results for the different trials are:

- at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

Laboratory tests (not required for product authorisation), simulated-use test:

- ≥ 80% attraction (number of released mosquitoes in relation to captured animals) within the test period according to the claim, from the beginning and until the end of the claimed efficacy period.

Field trials:

- ≥ 80% attraction within the test period according to the claim, from the beginning and until the end of the claimed efficacy period.

5.6.5.10 Stable flies indoor

Evaluation of *Stomoxys calcitrans* outdoor is part of the description for flies on grazing cattle, horses and other livestock.

5.6.5.10.1 Introduction

The stable fly (*Stomoxys calcitrans*) is a synanthropic fly of veterinary importance found worldwide. It is an obligate blood-feeder which prefers to feed on cattle, horses and pigs. In the absence of the primary hosts, they may bite humans as well as dogs. The bite is very painful and provokes considerable unrest, often causing defensive behaviour of the hosts when the host tries to get free of the stable flies. Horses and cattle, which are heavily infested may even become anaemic. It has been estimated that a decline of up to 25% in milk production can be attributed to stable flies dependent on the level of infestation. Additionally, the stable fly may act as a mechanical vector of various pathogens.

5.6.5.10.1.1 Biology

The stable fly, *Stomoxys calcitrans* L., (6-9 mm) is a medium size brown-greyish fly with an appearance not much different from a house fly (*Musca domestica*), both belonging to the family Muscidae. The two species may, on a superficial level, be confused with one another, as they are both very common in stables, cowsheds and pigsties. The house fly is a sucking fly not capable of penetrating the skin while the stable fly is a biting fly belonging to the subfamily Stomoxyinae (genus: Stomoxys) and without a doubt distinguishable from house flies by its long, slender prominent, forward-directed, pointed proboscis.

The stable fly is a cosmopolitan species. In the northern parts of Europe, the stable fly goes through its complete life-cycle in stables with livestock, especially cattle and pigs. On warm days, or in warmer southerly climates, flies may migrate between hosts in nearby fields; the breeding and mating take place on the farm. Both sexes are blood-feeding, needing frequent blood meals and usually take one blood meal per day. Stable flies favour blood from cattle, they also appreciate blood from horses and pigs as well and if forced they may bite other warm-blooded animals. Cattle and horses are in general attacked on the lower parts of the body and on the extremities, especially the forelimbs. Pigs are more often attacked behind the ears. During the dark hours, the stable flies are resting high in buildings, on ceilings and upper parts of the walls. Their main blood-seeking activity is highest between 10 am and 4 pm, but this is not very strict, and they may bite any time in the light hours if the conditions are suitable.

5.6.5.10.2 Dossier requirements

Dossier requirements are stated in the General Introduction (see section 5.6.5.1.3).

5.6.5.10.2.1 Test species

Tests with laboratory colonies of stable flies as well as with field collected specimens are applicable. In field experiments it must be clearly described how the test method ensured that only stable flies were counted in the experiment.

Products intended for use as repellents on domestic animals or as spatial repellents should be tested with the claimed host, e.g. cattle, horse or sheep. A general host claim is not acceptable.

Repellent testing should be performed with stable flies. It is not acceptable to use results from other fly species to support a claim for stable flies.

In case of specific claims, e.g. effective at high temperature, in contact with water, host sweating, etc., efficacy must be demonstrated in the relevant situations.

5.6.5.10.2.2 Requirement per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.10.2.2.1 Products intended for use as repellents

The efficacy of a product should be shown in either a robust simulated-use test, where animals can be tethered and observed close by, or a field trial where the animals are loose/untethered and observed from a distance to establish the efficacy and persistence of the product when used according to the label in a real-life situation.

For PT19 products which are applied on animals, no common protocols are available. However, the EMA has published several guidelines for the evaluation of ectoparasiticides, see Appendix 18. These guidelines may be adapted for the demonstration of the efficacy of repellent products to be applied on animals, but any well documented protocol can be used, provided it is scientifically sound.

It is important to give a detailed description of the circumstances in which the experiments are conducted, with special emphasis on the ratio of treated to untreated host animals at each trial location and trial replicate. Stable flies are forced to get blood meals, and therefore it may be of consequence for the trial outcome whether all hosts on a trial location are treated, compared to a situation where only a minor part of the hosts are treated leaving alternative untreated possibilities for the flies to get blood. It is also of importance that the diurnal rhythm of blood sucking activity of stable flies is taken into consideration in the setup of the efficacy trials and reported in detail. If the product is part of a push-pull strategy, then this setup must be reported in detail as well.

If treated horse rugs or similar covers are claimed to be effective in the stable, then it must be demonstrated effective in the stable. It is not important to make an evaluation of the flies on the rug itself, but the emphasis must be on the exposed parts of the host such as the neck, head, legs, and belly including the sheath on males and udder on females, which are thin-skinned areas and very attractive to several blood-feeding species.

Tests must be presented with all host animal species claimed. Especially in tests with horses, attention is drawn to the fact that horses react very sensitively to biting stable flies and often interfere with the counting as they try to avoid the biting flies. It may be necessary to make more repetitions with horses than with cattle or in other ways adjust the trial setup.

5.6.5.10.2.2.1.1 Simulated-use test

In a simulated-use test different parameters are controlled. A simulated-use test of a repellent with full control of host animals and absence of their normal avoidance behaviour can be used to evaluate the efficacy and the persistency of the treatment; topically, spatially or impregnated textiles. The product should be applied indoors according to the claim. Environmental conditions must be specified at the beginning and during the test (temperature,

humidity, photoperiod). No specific temperature or humidity are recommended, but the stable flies must be present in sufficient numbers, to be able to compare the treated and untreated group. A control treatment without repellent should be included in all trials to secure a measure of the activity.

A simulated-use test must be conducted indoor at a time in the season, as well as during the day where stable fly activity is sufficient in cowsheds, pigsty, etc. Depending on the target animal species, several factors (e.g. hair length, the thickness of the coat, grooming, etc.) might impact the efficacy of topical repellents. Explain in the test report/documentation the circumstances around the choosing of the test animals. If animals of different colour are chosen be sure not to have a majority of light-coloured animals. Ideally, light coloured animals are not included, but state on the label if the product is for a specific colour of animals, if only light coloured animals are tested a sound justification is needed to include all fur colours in the claim. For products only tested on e.g. pied (black or red) cattle, it can be necessary to state on the label that the products are for pied (black or red) cattle. For products tested on sheep it can be necessary to test on both sheared and unsheared sheep (a sound justification is needed if only sheared or unsheared sheep are tested).

At least two groups of host animals must be used, one group treated with the product and one group with no treatment. At least ten different individuals are suggested to be used in each group. In the case of horses, a setup could be 2x5 horses with a treatment switch after e.g. a week (depended on the residual efficacy of the tested product) tested at one location, or 2x5 animals tested at two different locations. At the end of the test period, there must be 2x10 valid datasets. Each treatment group should be housed in separate enclosures throughout the course of the trials to prevent cross infestation or cross-contamination.

Preferably, no test animals should have a recent history of treatment with insecticide/acaricide or antiparasitic agents. Concerning endo-antiparasitic agents this is acceptable, but details (product, dose, application rate, application method, date of the last treatment, and residual efficacy) must then be given in the report; the controls and the treated groups must have the same status. A safety margin for external applied anthelmintics is the end of last application + 1 month. The history of the treated and the control groups should be comparable.

Animals should be treated with the test product once the infestation has become established. The infestation should be documented by e.g. recordings of the numbers of flies, photographs. Collections or counts must be carried out in the same area on all hosts, typically on the legs. Inside barns and stables, it may be difficult to distinguish between *Musca domestica* and *S. calcitrans* at a distance and it is therefore acceptable that counts/collections are done on a tethered host. If the product is acting at a very close range it may be possible to make the evaluation by treating one hind leg and one front leg and then compare the difference in fly-load between the treated and untreated legs. The advantage of such a setup is the neutralisation of individual host differences in fly-attraction. The attractiveness of the test animals or experimental groups should be tested prior to treatment.

Percent inhibition (landing or feeding) is based on the number of stable flies landing/feeding on the untreated control animals (or room) and the number of stable flies landing/feeding on treated animals (room):

Pct inhibition: $((\text{LandingFeeding}_{\text{control}} - \text{LandingFeeding}_{\text{treated}})/\text{LandingFeeding}_{\text{control}}) \times 100$.

Alternatively or in addition to fly landing catches, also the frequency of certain horse's avoidance behaviour according to Mottet et al., see Appendix 18, can be recorded as a substitute, provided the abundance of the target species predominantly causing the avoidance behaviour is measured at the beginning and the end of the test.

5.6.5.10.2.2.1.2 Field trial

In a field trial the animals must have the opportunity to display normal avoidance behaviour considering how the product is intended to be used. The tests may be conducted as described for the simulated-use test above, with the adjustment that there is no interference with the host animal's normal behaviour in the stable.

The field trials should be conducted on two different geographical locations.

At least two groups of host animals must be used, one group treated with the product and one group with no treatment. At least ten different individuals are suggested to be used in each

group. Due to feasibility and cost-effectiveness, it would be possible to use 10 animals one week (5 each in the treatment and control groups) and then use the same individuals again in the second week, but swap treatments. Each animal would then act as its own control. However, any residual activity of the product has to be excluded. The field trials can be performed twice with 5 animals (in each of the control and treatment groups) each time at the same location, or with 5 animals (in each of the control and treatment groups) at two different locations. At least 10 animals (each as control and treatment) must be tested. The two locations can be in the same country or region, e.g. two villages. In the case of cattle, the test can be performed at only one location using 10 cows for the control and 10 cows for the treatment.

As documentation for persistency, counts are repeated with chosen intervals of lengths dependent on the claim. If there is a claim for use on wet/sweating hosts, then data must be provided showing that the product is effective under such circumstances. The guideline from the EMA: "Guideline on specific efficacy requirements for ectoparasiticides in cattle" (CVMP/625/2003), and Herholz et al. may be of inspiration for field trial setup, see Appendix 18.

5.6.5.10.2.2.2 Attractants without PT18 active substances

Use of an attractant in a trap in a push-pull setup may be suggested. A trap could be loaded with CO_2, heat, octenol, butyric acid, black colour ball, etc. In such a setup the fly load will be measured as described above.

For product authorisation purposes, the efficacy of an attractant in a trap without an active substance according to PT18 should be proven in a simulated-use test or a field trial according to the instructions for use.

The attractants, e.g. the traps, used for the test should be identical to the product to be marketed (see Introduction, section 5.6.5.1.3.7). Traps should be tested on their own, with a control tested separately in a similar test setting.

5.6.5.10.2.2.3 Attractants in combination with PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.10.3 Assessment of authorisation

5.6.5.10.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for stable flies on livestock in the following way:

<u>Products intended for use as repellent on animals (including a delivery system)</u>

A simulated-use test or a field trial demonstrating ≥80% repellency within the test period (according to the claim), directly after product application and at the end of the claimed use period.

All claimed target host animals must be tested.

In case of specific claims (e.g. effective at high temperature, in contact with water, host sweating, etc.), these should be demonstrated.

<u>Products intended for use as a space repellent or for use as attractants without PT18 active substances</u>

A trap with an added attractant should catch significantly more stable flies than one without the attractant. The traps used for the test should be identical to the product to be marketed.

A simulated-use test or a field trial demonstrating ≥ 80 % efficacy within the test period according to the claim, directly after product application and at the end of the claimed use period.

For attractants at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

All claimed target host species must be tested.

The product label and SPC should state that the entire target insect population might not be caught/repelled by this type of product; the label claim should be "reduces" and not "protects"/"protection", with no mention of a CPT.

5.6.5.11 Stored goods-attacking insects and mites

5.6.5.11.1 Introduction

The main purpose of biocidal products against stored goods-attacking and infesting insects and mites is to:

- control pests in storerooms, freight and alternative transport containers for products of plant origin or for other goods;
- protect the actual stored goods against damage or contamination by insects and mites;
- disinfest empty storerooms.

Products related to product-type PT19 in this case are repellents and attractants used to control harmful stored-products pests by repelling or attracting, e.g. mass trapping, i.e. reducing target organism numbers by luring them in large numbers to a trap that contains an attractant and killing the target organisms either with a biocide (attract-and-kill, PT18 product) or a devise that bars their exit (PT19 product), or mating disruption.

The biocidal products are mainly used to achieve hygiene, consumer protection and food-/feed-/material- or stored goods-protection. The term "stored" in this regard refers specifically to stored products for human use and consumption, and animal feed and industrial processing. During storage, transportation, processing and trade stored goods are exposed to many hazards. Biological threats such as insects and mites cause feeding damage and contamination. Contamination may be caused by living and dead mites, beetles and moths, their larvae and eggs as well as mould growth as a secondary infestation. This may lead to spoilage or reduction in quality and imply a health risk for humans and animals. An infestation is often difficult to detect because the organisms are very small, and some develop inside the goods. Sometimes the infestations are only noticed by the consumer once the insect leaves e.g. the food product and enters the home environment. Therefore, in industrial premises monitoring traps are common to detect an infestation at the initial stage.

Biocidal products against stored goods-attacking and infesting insects and mites are applied in processing facilities and enterprises, along the supply chain and at the consumer site, e.g. uses in storerooms, warehouses, food/feed industry, private households, public buildings, cargo compartments on ships or airplanes and other transport containers for processed products. Application can take place in empty as well as in stocked rooms depending on the intended use.

Regarding the control of stored-goods attacking insects and mites it has to be mentioned that protection of goods of plant origin is a borderline case: products against stored goods-attacking insects can be categorized as either biocides or plant protection products depending of the purpose of the intended use. In general, uses controlling harmful organisms in unprocessed plant products of plant origin or those which undergo only simple preparation fall under the regulations of plant protection, unless the uses are considered to be for reasons of hygiene rather than for the protection of plants or plant products (EC/1107/2009). Aspects of plant protection and plant protection products are not covered by this guideline, but the control of insects and mites for reasons of food/feed security, processed goods and materials hygiene is.

Compared to repellents the use of attractants against stored goods-attacking insects and mites in stored-product protection is already common, e.g. dispensers in bakeries or pheromone moth-traps in households.

5.6.5.11.1.1 Target and test organisms

Among the organisms attacking stored goods certain groups of organisms are of particular importance: beetles (especially weevils), moths, dust-lices and mites. Some of the most common pests that infest stored plant products on their way from primary production via processing industry, transportation and trade to the consumer are listed and suggested as test organisms in EPPO guidance. EPPO focuses on insects and mites known as typical harmful organisms of stored-plant products. However, these arthropods could also infest processed and packaged products like cereal mixtures, dried fruits and vegetables, processed cereal products,

bakery products, chocolate, confectionary and pasta, expeller, nuts, pulses, oilseeds, herbs, tea etc. Some of them, e.g. *Dermestidae* (especially their larvae) could also live or exist in dried products of animal origin like milk powder and meat.

This is a non-exhaustive list of relevant target organisms:

Beetles (including weevils) such as:

Acanthoscelides obtectus – Bean weevil

Alphitobius diaperinus – Lesser mealworm / litter beetle

Anobium punctatum – Common furniture beetle

Anthrenus flavipes – Furniture carpet beetle

Anthrenus munroi

Anthrenus museorum – Museum beetle

Anthrenus pimpinellae – Bird nest carpet beetle

Anthrenus scrophulariae – Buffalo carpet beetle

Anthrenus verbasci – Carpet beetle

Attagenus unicolor – Black carpet beetle

Callosobruchus maculatus – Cowpea weevil

Cryptolestes ferrugineus – Rusty grain beetle

Cryptolests busillus – Flat grain beetle

Dermestes lardarius – Larder beetle

Hylotrupes bajulus – House longhorn beetle

Lasioderma serricorne – Tobacco beetle

Lyctus brunneus – Brown powderpost beetle

Oryzaephilus surinamensis – Sawtoothed grain beetle

Rhyzopertha dominica – Lesser grain borer

Sitophilus granarius – Wheat weevil

Sitophilus oryzae – Rice weevil

Sitophilus zeamais – Maize weevil

Stegobium paniceum – Drugstore beetle

Tenebrio molitor – Yellow mealworm

Tenebroides mauritanicus - Cadelle

Tribolium castaneum – Red flour beetle

Tribolium confusum – Confused flour beetle

Trogoderma granarium – Khapra beetle

Trogoderma inclusum – Larger cabinet beetle

Trogoderma longisetosum

Trogoderma variabile

Moths such as:

Cadra cautella (*Ephestia cautella*) – Almond moth / tropical warehouse moth

Corcyra cephalonica – Rice moth

Endrosis sarcitella – White-shouldered house moth

Ephestia elutella – Cacao moth/Tobacco moth

Ephestia kuehniella – Mediterranean flour moth

Hofmannophila pseudospretella – Brown house moth

Nemapogon granella – European grain moth

Plodia interpunctella – Indian meal moth

Pyralis farinalis – Meal moth

Sitotroga cerealella – Angoumois grain moth

Booklice/dustlice such as:

Liposcelis corroden

Liposcelis decolor

Dorypteryx domestica

Mites such as:

Acarus siro – Flour mite

Lepidoglyphus destructor – Storage mite

Tyrophagus longior – Mould mite

Tyrophagus putrescentiae – Cheese mite

5.6.5.11.2 Dossier requirements

General dossier requirements are stated in the General Introduction (see section 5.6.5.1.3).

5.6.5.11.2.1 Test species and conditions

The (groups of) organisms included in the group of stored goods-attacking insects and mites do not belong to a single monophyletic group, but to different classes (insects and mites (arachnida)) or orders, e.g. beetles and booklice. Therefore, general claims at such a high level are difficult to substantiate. In practice, products for use against stored goods-attacking insects and mites will typically cover the control of pests for a specific type of stored goods, e.g. flour, grain, tobacco, processed cereals, paper, leather.

Due to the diverse nature (biology, ecology, habitat, diet, behaviour, etc.) of the organisms included in the group of stored-goods attacking insects and mites, and due to the specificity of active substances such as pheromones, efficacy can only be claimed against the species for which efficacy was demonstrated in efficacy studies. Nevertheless, general claims for smaller groups which consist of specific organisms and a specific type of stored good to be protected could be authorised. This is the case, for example, when different moth species may produce the same pheromone components for the attraction of sex mates. Semiochemicals are often pest specific and act by modifying behaviour. In the case semiochemicals that have multiple targets, extrapolation to a group of related species is possible. A schematic representation of the extrapolation possibilities on effectiveness is available in the EPPO standard PP1/296. Alternative extrapolations may be proposed by the applicant. A clear justification is always necessary and may be supported by scientific literature and/or data. For semiochemicals EPPO Standard PP1/264 has specific advice on mating disruption pheromones, including a schematic representation of extrapolation possibilities given in PP1/296. For extrapolation from efficacy data for one species to a limited group a robust justification based on scientific literature and/or data should be provided.

Table 37: Examples of different pheromones that are used to attract food moths.

Group of target organisms	Pheromone	Species against which efficacy should be demonstrated
Food moths or storage moth species (e.g. processed cereals, dried fruits, flour, processed nuts, infusions) – Pyralidae	(Z, E)-9,12-tetradecadien-1-yl acetate (TDA)	*Ephestia kuehniella* *Ephestia elutella* *Plodia interpunctella*
Food moths or storage moth species (e.g. processed cereals, dried fruits, flour, processed nuts, infusions) – Gelechiidae	(Z, E)-7,11-hexadecadien-1-yl acetate (HDA)	*Sitotroga cerealella*

For general claims, apart from efficacy studies, a justification based on robust scientific information, e.g. scientific literature, has to be provided, demonstrating efficacy against the organisms covered by the claim, e.g. by demonstrating all moth species covered by the claim use the same pheromone.

General claims should include both the group of organisms, e.g. a specific genus of beetles, moths, to be controlled and the type of stored goods to be protected. If multiple general claims are made, efficacy should be demonstrated for each of these claims. Examples of possible general claims and the species against which efficacy should be demonstrated are presented in the tables below.

The mentioned target groups, species and goods to be tested or protected are only examples. It is advised to discuss with the competent authority the acceptability of any general product claims, with regard to target organisms or goods to be protected, prior to product dossier submission.

Table 38: General claims

General claims based on target organisms

Group	Species against which efficacy should be demonstrated
Flour moths	*Ephestia cautella* *Plodia interpunctella or Ephestia kuehniella*
Grain (products) beetles	*Cryptolestes busillus* or *Cryptolestes ferrugineus* *Oryzaephilus surinamensis* *Tenebroides mauritanicus or Tenebrio molitor*
Grain weevils	*Sitophilus granarius* *Sitophilus zeamais or Sitophilus oryzae*

General claims based on goods to be protected

Good to be protected	Species against which efficacy should be demonstrated
Stored grain	*Sitophilus granarius* *Rhyzopertha dominica* *Oryzaephilus surinamensis* *Cryptolests busillus or Cryptolestes ferrugineus* *Sitophilus granarius* *Sitophilus zeamais or Sitophilus oryzae* *Tenebroides mauritanicus or Tenebrio molitor* *Tribolium sp.* *Plodia interpunctella or Ephestia kuehniella* *Sitotroga cerealella*
Flour and flour products	*Tribolium* sp. *Oryzaephilus surinamensis* *Sitophilus zeamais* or *Sitophilus oryzae* *Tenebroides mauritanicus or Tenebrio molitor* *Plodia interpunctella or Ephestia kuehniella* *Trogoderma granarium* *Lasioderma serricorne* *Stegobium paniceum* *Ephestia kuehniella*
Spices and Tobacco	*Lasioderma serricorne* *Stegobium paniceum* *Dermestes* sp.
Dehydrated foods	*Plodia interpunctella* *Dermestes* sp. *Trogoderma granarium*
Nuts and dried fruit	*Plodia interpunctella or Ephestia cautella* *Oryzaephilus surinamensis* *Tribolium* sp.
Dried peas and beans	*Acanthoscelides obtectus* *Callosobruchus maculatus*

The applicant has to describe the target organisms and any special details depending on the species and relevant for the efficacy of the biocide, e.g.:

- Sex and status of used insects/mites;
- Nature of communication;
- Developmental stages (eggs, larvae, pupae, diapausing larvae, imagines);
- Mating/calling behaviour and season, polygamous males;

- Special characteristics like flying in dawn light, living/developing inside the cereal, egg laying sites, seasonal aspects, movement distances of adults;
- Resistance risk and appropriate management strategies, if relevant (see also EPPO guidance).

With regard to test conditions, the following aspects, if relevant, should be considered:

- Test room/space dimensions should be representative of intended use;
- Information on the used methods should be provided (e.g. mass-trapping, mating disruption, "attract and kill");
- Is the intended use to attract or repel one sex or both;
- Devices (e.g. spraying cans / traps) used to apply the product in efficacy tests should be the same as for the product to be authorised (specify design, color and position in test setup);
- Prevent interference between treatment rooms/plots;
- Treatment with different dosages and pest densities, if relevant;
- Climate data should be recorded: e.g. air dynamics in the test facility, temperature (range of change and or monthly mean), humidity (range of change and/or monthly mean). Climatic conditions should be in accordance with the intended area of use.

For pheromone attractants:

- There may not always be a good correlation between trap catches and subsequent infestation/crop damage when using the same sex pheromone for monitoring traps and mating disruption:
  - males could not locate traps in a pheromone area, or;
  - they could be back-caught while the dispensed pheromone level is too low;
  - deflect attention from females;
  - alternative approach: traps with other semiochemicals (kairomones) for the trap lures, light traps or water traps (for *Ephestia* moths);
- Development of the population of the pest organism (depends on reproduction, nutrition, environmental conditions) should be monitored;
- Avoid immigration of mated females to avoid test disturbance by reproduction. For simulated-use test virgin females should be used, if relevant.

Please note that the parameters above are examples, the relevance of each of these is dependent on the product to be authorised and claim(s).

5.6.5.11.2.2 Requirements per type of claim and test methods

In the product claim, it should be specified whether the product is intended to treat an ongoing infestation or to prevent new infestations. Depending on the intended use, e.g. the application and the purpose of the product testing requirements are listed in sections 5.6.5.11.2.2.1 and 5.6.5.11.2.2.2. For both simulated-use tests and field studies, a minimum of 5 valid replicates (different test sites/locations/buildings in the case of a field study) is required. For each claimed condition of use (indoor, outdoor, cupboard, storage room) 5 replicates should be performed.

A laboratory test is not required for product authorisation but can be used to evaluate if the right dose is chosen and to see whether the product repels under ideal conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

For simulated-use tests with sex pheromones, both sexes should be released as in nature the traps will compete with the pheromone released by the females. A food source should be present when measuring reproduction (F1 generation) and/or when reinfestation of stored goods is relevant for the label claim.

For simulated-use tests with alimentary attractants, an alternative food source should be offered in the test setup. The time needed to reach the claimed efficacy has to be indicated.

Due to the diverse nature (biology, ecology, habitat, diet, behaviour, etc.) of the organisms included in the group of stored-goods attacking insects and mites, and due to the specificity of active substances such as pheromones, efficacy can only be claimed against the species for which efficacy was demonstrated in efficacy studies. Only developmental stages against which efficacy was demonstrated can be authorised. For a general claim additional data needs to be provided, e.g. from the scientific literature, demonstrating the acceptability of the claim.

In the simulated-use tests, the number of test organisms should be proportional to the room size (relevant pest density) and sufficient to draw statistically robust conclusions.

5.6.5.11.2.2.1 Products for consumer (non-professional) use

For consumer products, simulated-use tests are required. Simulated-use tests can be waived if a robust field trial is submitted. A simulated-use test can be a test, performed in a laboratory, where insects or mites (either cultured or natural populations) are in contact with the stored goods, e.g. breakfast cereal, flour) and the biocide is applied according to the instructions for use.

A control treatment in parallel without biocide with the same number of replicates should be included in all simulated-use trials. Tests should be performed in space/room of a size relevant for the intended use.

5.6.5.11.2.2.2 Products for professional use

For products for professional use field trials are required. Given the sometimes large and complex environments, e.g. warehouses, where products are to be used, field trials, e.g. on existing infestations or with known numbers of test organisms and stages are mandatory when it comes to demonstrating efficacy, especially in the case of mating disruption. For field trials, trials should be conducted under representative conditions, e.g. food moth in food factory, or insect pest of animal or vegetable product in the storeroom, etc. In case residual efficacy is claimed, efficacy data should be submitted to support the claimed residual efficacy period after product application (opening of the product).

5.6.5.11.2.2.3 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.11.3 Assessment of authorisation

5.6.5.11.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". Indicators of effectiveness for stored goods-attacking insects and mites are, e.g.:

- Population reduction;
- Control of population target (e.g. sex, developmental stage): male or female catches in relation to the released vital population; behaviour of untrapped /remaining males (e.g. impaired in the finding capability);
- Damage of products, population reservoir (if known).

Moderate effectiveness may not be acceptable because of the risk of consolidating the infestation and the carry-over with traded goods.

This is implemented for stored goods-attacking insects and mites in the following way:

Attractants

- ≥80% population target, e.g. sex, developmental stage, attraction compared to the negative control for laboratory, simulated-use and field trials. Efficacy should be demonstrated within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period;

- at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

For products intended for use as attractant in combination with PT18 (bait products), the efficacy evaluation should be done on the basis of the requirements for products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

Repellents

- ≥80% repellency (influence on population dynamics, population reduction, influence on population target) for laboratory, simulated-use and field trials. Efficacy should be demonstrated within the test period (or according to the claim), at the beginning and until the end of the claimed efficacy period.

Proof of non-insecticidal efficacy has to be examined in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8). After the trial insects are maintained in the test system to observe whether insecticidal effects could be caused by contact/exposure to the test repellent product. Mortality of the insects/mites should be monitored after 24 hours.

Deviations from these norms are possible but should be justified in the application, e.g. data on protection of the stored goods may be used if 100% protection was demonstrated.

5.6.5.12 Textile-attacking insects (including fur and fabric attacking insects)

5.6.5.12.1 Introduction

The purpose of biocidal repellents or attractants against textile-attacking insects is to prevent or to combat pest infestation of textile articles especially those manufactured from natural fibres like wool, hair, feathers or fur and also blends of natural and synthetic fibres.

Repellents against textile-attacking insects can also be incorporated in the textile by industry for preventive treatments.

5.6.5.12.1.1 Biology

The two main orders containing textile-attacking insect species are Lepidoptera (moths) and Coleoptera (beetles). The webbing clothes moth (*Tineola bisselliella*), case-making clothes moth (*Tinea pellionella*), brown house moth (*Hofmannophila pseudospretella*) and carpet beetles (*Anthrenus* spp., *Anthrenocerus* spp., *Attagenus* spp., *Trogoderma* spp.) are common in-house pests that feed on clothing, drapery, carpet and other natural hair fibres. The larvae of these insects feed on natural hair fibres, which provide protein from keratin in the hair. They have adapted to be able to digest keratin, which is not easily digested by other insects.

Clothes moths are distributed worldwide. They feed during the larval cycle either within a silken cocoon attached to hair fibre (only *T. bisselliella*) or inside a portable silken case (all other textile moth species). Clothes moths larvae that feed only on natural hair fibres such as wool, will not feed on pure silk, pure cotton, pure linens or pure synthetic fibres. Adult clothes moths do not feed. After mating the females lay eggs directly on the natural fibre food source.

Carpet beetle larvae, e.g. *Anthrenus* sp., *Anthrenocerus* sp., *Attagenus* sp. attack woollens, rugs and upholstered furniture, among other things. The adult beetles, which feed on nectar and pollen, can enter the home on plants, flowers or other vegetation. Bird nests and animal burrows which may be found in or close to houses may also provide an entry path. In the context of museums there is likely an entrance through artefacts brought in from other museums with an insect pest problem. Eggs are then laid on lint in protected areas such as behind baseboards. Once hatched, larvae begin feeding on a number of natural textiles or displays (animal horns, hoofs, insect collections, etc.).

5.6.5.12.2 Dossier requirements

Dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

5.6.5.12.2.1 Test species

A product against textile-attacking insects should be tested on:

At least one of the following moth species:

- the webbing clothes moth (*Tineola bisselliella*)

- the case-making clothes moth (*Tinea pellionella*)
- the brown house moth (*Hofmannophila pseudospretella*)

At least one of the following carpet beetle species:

- *Anthrenus* spp.
- *Attagenus* spp.
- *Anthrenocerus* spp.

For a general claim "against adult textile-attacking insects" adult target organisms, moths and carpet beetles, should be tested. For general claims "against adult textile-attacking moths" or "against adult textile-attacking carpet beetles" adult moths or carpet beetles should be tested, respectively. If products are exclusively claimed against larvae of moths and/or carpet beetles, then the efficacy should be demonstrated for that developmental stage (instead of adults). Deviations should be justified and tested.

Due to the specificity of certain active substances, e.g. pheromones, for products based on an active substance with a species-specific mode of action, only effects against textile-attacking insect species that have been tested under simulated-use and/or field conditions, depending on the type of claim, should be claimed on the product label and in the SPC.

The composition of the material to be protected should be indicated on the label, to avoid application of products on materials which are not attractive to textile-attacking moths and/or carpet beetles.

5.6.5.12.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.12.2.2.1 Repellent products against textile attacking insects

In most cases, these products will not be able to eliminate an existing infestation. They are only useful as a preventive measure. Unless otherwise proven in efficacy trials, the label should include wording such as: "Ensure there is no infestation in place before the use of this repellent. Repellents should only be used as a preventive measure".

For product authorisation purposes the efficacy of repellent products should be proven in a simulated-use test according to the instruction for use (test design examples see 5.6.5.12.2.2.1.3 and 5.6.5.12.2.2.1.4).

The repellents used for the test should be identical to the product to be marketed.

Proposed label claims regarding the performance of the product must be simulated in the study (for details see Introduction, section 5.6.5.1.3.5).

For products intended to be used as space treatment the room size stated in the SPC and on the label must be used in the efficacy trial. For the extrapolation to larger room sizes than proven in the efficacy test a justification is necessary.

5.6.5.12.2.2.1.1 Laboratory choice test for repellents

The repellence of an active substance can be tested in a choice test, examples see Appendix 18: Beerwinkle K.R., et al. and Arnault I., et al.. Insects are placed in a tunnel or a release box interconnected with tunnels between two dark boxes, both containing fabric made of a material which is in accordance with the claim. One of the boxes contains the repellent product. The application quantity of the product must match the size of the boxes. The insects (at least 10) are released in the tunnel or release box, then they can choose the treated or control box. The ratio of insects found in the treated vs. control box is a measure of the efficacy of the product. Insects in the tunnel that have not chosen a side (control or treated box) should not be used in the evaluation of the product. Saturation of the system with repellent should be avoided. If necessary, the system should be ventilated, e.g. air has to be sucked out at the insect's release site and introduced both at the treated and control box. If a product is claimed against one sex

an equal number of both sexes can be used in the test, but only the target sex should be evaluated.

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.

Environmental conditions must be specified (and justified) for the test itself, and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation) and shall be in accordance with the claim. Please note, the darker the test conditions, the better the insects behave and the more realistic the experiments are.

5.6.5.12.2.2.1.2 Laboratory choice test for oviposition repellent products

A choice test similar to the test described in section 5.6.5.12.2.2.1.1 can be used with the following adaptations:

The dark boxes contain fabric attractive for insect oviposition. The nature of the fabric should be in accordance with the claim and the fabric should be attractive to the textile-attacking insects for oviposition. The attractivity of the fabric must be documented.

To record the development of potentially laid eggs during the test period the fabrics are placed in closed trays and incubated. Breeding conditions must be specified and justified for the test itself and shall be in accordance with the claim. Please note, the darker the test conditions, the better the insects behave and the more realistic the experiments are. The degree of repellence is shown as the number of eggs on the treated fabric versus the number of eggs on the control fabric or alternatively, the number of offspring (larvae) hatched from the treated fabric versus the offspring hatched from the control fabric.

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.

5.6.5.12.2.2.1.3 Simulated-use test for repellents

Mandatory requirements:

- The simulated-use test should reflect the real use situation.
- Simulated-use tests should be conducted in closets. If the product is claimed for an application in closets a minimum volume of 0.5 m^3 should be used, which may be divided into smaller compartments. For products to be used in smaller compartments, like drawers or boxes, tests should be done in containers with a volume according to the claim appearing on the SPC.
- The treatment and control group tests are conducted each in a separate room. The control serves to show that the test insects are vital and able to infest the closet under test conditions.
- The degree of repellence is shown by the number of test insects (including the potential offspring) in the test closet versus the number of test insects (including the potential offspring) in the negative control closet.
- Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed. Each replicate consists of at least 50 mature clothes moths or carpet beetles.
- Environmental conditions must be specified (and justified) for the test itself, and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation) and shall be in accordance with the claim. Please note, the darker the test conditions, the better the insects behave and the more realistic the experiments are.
- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).
- For the evaluation of the claim “protects clothing” damage to the test material must be documented by photography. Compared to the control setup no damage to the textiles of the treated cabinet should be documented.
- For residual efficacy, textile-attacking insects are exposed to the product at several time intervals after application (including the end of the claimed period).

Other test designs than the following example (see Appendix 18: Plarre R., et al.) can be accepted if the protocol is scientifically valid. Deviations from this test design, e.g. number of test insects in a lower or higher volume in adoption to the intended uses are possible and must be explained.

Test design:

One closet is located centrally in a ventilated test room (Peet Grady chamber or equivalent ventilated room with a size of at least 1.80 x 1.80 x 1.80 m). To mimic the practical use situation pieces of fabric that are attractive to insects and which nature is in accordance with the claim should be placed inside the closet. Furthermore, the door of the closet should be opened with a frequency resembling the normal opening of a closet, once a day for at least 10 seconds, to show that this does not reduce efficacy during the completion of the assay. The closet should provide openings, e.g. a number of holes of 2-4 mm diameter or a longer crevice with 2-4 mm width, for entry of the moths or beetles when the door of the closet is closed.

Sample trays filled with pieces of biocide-free attractive fabrics in accordance with the claim are deposited without a cover on 2-3 different compartments of the closet each with at least one tray. These open test trays are used to monitor egg deposition and offspring development of the target organisms. Products should be applied according to the claim (dose, application method). The attractivity of the fabric must be documented. No food source should be added with the biocide free samples. This could bias the results, or if feeding is necessary then laboratory food could be provided in both the treated and control samples.

At least 50 adult mature clothes moths or carpet beetles are released approximately 1 m in front of the closet door into the test room and monitored at regularly defined time intervals until the end of the claimed efficacy period (residual efficacy). At each time interval, the test insects should be monitored for several days after releasing into the room, e.g. 5 days after the introduction of the product. Test insects can move freely within the test room. Access into the closet should be possible.

At the end of the test-run, insects inside and outside the closets are collected separately. If a product is claimed against one sex an equal number of both sexes can be used in the test, but only the target sex should be evaluated.

The chamber and the closet must be cleaned and dried between each replicate to avoid chemical contamination and saturation of the product.

Proof of non-insecticidal efficacy: After product application test insects should be placed in the test closet (or smaller compartments) for 1 hour to observe whether insecticidal effects could be caused by contact/exposure to the test repellent product. To avoid a no-choice test which forces the insects into contact with the product, the insects should be able to avoid direct contact with the product. After each test run, the mortality of the adult insects should be monitored.

Efficacy assessment: To record the development of potentially laid eggs during the test period the sample trays are covered and incubated. Breeding shall be conducted until the development of eggs to adults is completed in the control group.

The rate of efficacy of a product depends on its capability of preventing textile-attacking insects from entering the closet. The repellent effect (%) is calculated by comparing with the negative control (insects inside the control closet, number of offspring).

5.6.5.12.2.2.1.4 Simulated-use test for oviposition repellent products

A simulated-use test similar to the test described in section (5.6.5.12.2.2.1.3) can be used with the following adaptations:

At the end of the test run only the development of potentially laid eggs during the test period must be recorded. The number of insects inside and outside the closets must not be determined. The degree of repellence is shown as the number of eggs on the treated fabric versus the number of eggs on the control fabric or alternatively the number of offspring (larvae) hatched in the test tray versus the offspring hatched in the control tray.

5.6.5.12.2.2.2 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractant products intended for use in traps should be proven in a simulated-use test according to the direction of use (test design example see 5.6.5.12.2.2.2.2).

The attractants, e.g. the traps, used for the test should be as similar as possible to the product to be marketed (for details see Introduction, section 5.6.5.1.3.7).

5.6.5.12.2.2.2.1 Laboratory choice test for attractants

Attractiveness of an active substance can be tested in a choice test, examples see Appendix 18: Beerwinkle K.R., et al. and Arnault I., et al. Insects are placed in a tunnel or a release box interconnected with tunnels between two dark boxes, both containing fabric made of a material which is in accordance with the claim. One of the boxes contains the attractant product. The application quantity of the product must match the size of the boxes. The insects (at least 10) are released in the tunnel or release box, then they can choose the treated or control box. The ratio of insects found in the treated vs. control box is a measure for the efficacy of the product. Insects in the tunnel that have not chosen a side (control or treated box) should not be used in the evaluation of the product. Saturation of the system with attractant should be avoided. If necessary, the system should be ventilated, e.g. air been sucked out at the insect's release site and introduced both at the treated and control box. If a product is claimed against one sex an equal number of both sexes can be used in the test, but only the target sex should be evaluated.

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed.

Environmental conditions must be specified (and justified) for the test itself, and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation) and shall be in accordance with the claim. Please note, the darker the test conditions, the better the insects behave and the more realistic the experiments are.

5.6.5.12.2.2.2.2 Simulated-use test for attractants

Mandatory requirements:

- The simulated-use test should reflect the real use situation.
- Simulated-use tests should be conducted in closets. If the product is claimed for an application in closets a minimum volume of 0.5 m^3 should be used, which may be divided into smaller compartments. For products to be used in smaller compartments, like drawers or boxes, tests should be done in containers with a volume according to the claim appearing on the SPC.
- The treatment and control group tests are conducted each in a separate room. The control serves to show that the test insects are vital and able to infest the closet under test conditions.
- Attraction depends on the mode of action of pheromones by the attraction of adult individuals of a specific sex or by the attraction of males and females (sex-unspecific). Therefore, the degree of attractants could be shown for sex-unspecific products as the trapped number of test insects in a closet containing the trap with the attractant versus the trapped number of test insects in a control closet containing the identical trap without the active substance. For products which affect only individuals of one sex, the efficacy should be shown on the next generation, i.e. by counting eggs, larvae or pupae on fabrics in the treated and control closet.
- Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed. Each replicate consists of at least 50 mature clothes moths or carpet beetles.
- Environmental conditions must be specified (and justified) for the test itself, and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation) and shall be in accordance with the claim. Please note, the darker the test conditions, the better the insects behave and the more realistic the experiments are.

- Non-insecticidal efficacy must be proven, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).
- For the evaluation of the claim "protects clothing" damage to the test material must be documented by photography. Compared to the control setup no damage to the textiles of the treated cabinet should be documented.
- For residual efficacy, textile-attacking insects are exposed to the product at several time intervals after application (including the end of the claimed period).

Other test designs than the following example (see Appendix 18: Plarre R., et al.) can be accepted if the protocol is scientifically valid. Deviations from this test design, e.g. number of test insects in a lower or higher volume in adoption to the intended uses are possible and must be explained.

Test design: One closet is located centrally in a ventilated test room (Peet Grady chamber or equivalent room with a size of at least 1.80 x 1.80 x 1.80 m). To mimic the practical use situation pieces of fabric that are attractive to insects and which nature is in accordance with the claim should be placed inside the closet. Furthermore, the door of the closet should be opened with a frequency resembling the normal opening of a closet (once a day for at least 10 seconds), to show that this does not reduce efficacy during the completion of the assay. The closet should provide openings, e.g. a number of holes of 2-4 mm diameter or a longer crevice with 2-4 mm width) for entry of the moths or beetles when the door of the closet is closed.

Sample trays filled with pieces of biocide-free attractive fabrics in accordance with the claim are deposited without a cover on 2-3 different compartments of the closet each with at least one tray. Products should be applied according to the submitted claim on the SPC (dose, application method). The attractivity of the fabric must be documented. No food source should be added with the biocide free samples. This could bias the results, or if feeding is necessary then laboratory food could be provided in both the treated and control samples.

At least 50 adult clothes moths or adult carpet beetles are released into the closet. After an acclimatization period pieces of fabric are replaced, and the trap is applied in accordance with the claim appearing on the SPC.

Number of trapped insects is regularly monitored at defined time intervals until the end of the claimed efficacy period (residual efficacy). At each time interval, the test insects should be monitored for several days after releasing into the room, e.g. 5 days after the introduction of the product. A mixed population can be released, but just the target sex should be evaluated.

The chamber and the closet must be cleaned and dried between each replicate to avoid chemical contamination and saturation of the product.

Efficacy assessment: The rate of efficacy of a product depends on its capability to attract textile-attacking insects. The attraction (%) is calculated by comparing with the negative control (by counting the number of adult insects in the trap or by the number of offspring).

5.6.5.12.2.2.3 Attractants in PT18 bait products

Efficacy evaluation of such products should be done on the basis of the requirements for bait products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.12.3 Assessment of authorisation

5.6.5.12.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for textile-attacking insects in the following way:

<u>Products intended for use as repellent against textile-attacking insects</u>

Non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

Laboratory tests (not required for product authorisation):

- ≥ 80% repellence of adults and larvae within the test period according to the claim (sex, developmental stage, type of tissue, etc.) and until the end of the residual period;
- ≤ 20% offspring compared to 100% of control group;

The required results for simulated-use tests are:

Claim "protect textiles" or "prevents larvae from feeding"

- 100% textile protection documented by photography (i.e. no damage on textiles in the test) within the test period according to the claim and until the end of the residual period;
- ≥ 80% repellence of adults and larvae within the test period according to the claim (sex, developmental stage, type of tissue, etc.) and until the end of the residual period;
- in the treated closets ≤ 20% offspring compared to 100% of control group.

Claim "effective against textile-attacking insects"

- ≥ 80% repellence of adults and larvae within the test period according to the claim (sex, developmental stage, type of tissue, etc.) and until the end of the residual period;
- in the treated closets ≤ 20% offspring compared to 100% of control group.

Attractants without PT18 active substances

The required results for simulated-use tests are:

- ≥ 80% attractiveness compared to the negative control within the test period according to the claim (sex, developmental stage, type of tissue, etc.), from the beginning and until the end of the claimed efficacy period;
- for sex specific attractants: ≥ 80% attractiveness for the receiving sex;
- at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

5.6.5.13 Ticks

5.6.5.13.1 Introduction

Ticks (order: Ixodida), along with mites belong to the subclass Acarina (also known as Acari) within the class Arachnida. All ticks are obligatory blood-sucking parasites, and certain tick species can carry and transmit a variety of different pathogenic microorganisms including bacteria, viruses, protozoa and fungi. The most important tick vector species in Europe is *Ixodes ricinus*. Diseases vectored by this tick include Lyme disease (also known as Lyme borreliosis), tick-borne encephalitis (TBE), rickettsiosis, babesiosis, and anaplasmosis, which can affect both humans and animals. The tick *Hyalomma marginatum* is a vector of the Crimean-Congo haemorrhagic fever virus (CCHF), in Europe mainly restricted to the eastern Mediterranean area (Turkey and the Balkan region), Spain and the southern parts of France and Italy. The Mediterranean spotted fever, a rickettsial disease, is transmitted by the brown dog tick, *Rhipicephalus sanguineus*. This tick occurs mainly in buildings where dogs live and can transmit a number of pathogens. Further abundant species are *Dermacentor marginatus* that occurs predominantly in the Mediterranean climate zone, and *Dermacentor reticulatus*, occurring in slightly cooler areas. The latter is a known vector of *Babesia canis*, causing canine babesiosis, and both can transmit rickettsia spp.

PT19 biocides against ticks can only claim to repel or attract (in order to trap) the target organisms, not to prevent the diseases.

5.6.5.13.1.1 Biology

Ticks are characterized by two main body parts and eight legs as nymphs and adults, as opposed to insects, which have three main body parts and six legs. Ticks go through four stages to complete their lifecycle: egg, six-legged larva, eight-legged nymph, and adult. Feeding will occur in both the immature and adult stages.

Except one African species (*Nuttalliellia namaqua*), all ticks can be classified into two families: hard ticks (Ixodidae) and soft ticks (Argasidae). Key differences are that soft ticks lack the sclerotized scutum (dorsal shield) apparent in hard ticks and that the mouthparts of soft ticks are located ventrally and cannot be seen from above, in contrast to hard ticks having their mouthparts located distally, thus being easily visible from above.

There are also differences in the life cycle: most soft ticks develop through several nymphal instars (usually 2-4), and the adult female tick can repeatedly feed blood (usually 2-5 times) and typically lay between 100 and 300 eggs after each blood meal, which most often lasts between 20 and 50 minutes. In hard ticks, there is only one nymphal instar and the adult female tick only feeds once, followed by oviposition of hundreds or up to thousands of eggs. The blood meal of hard ticks lasts between 2 and 8 days, depending on species and stage. During that time, the tick is fixed to the host`s body.

There are two basic host-seeking strategies in ticks: the hunting and the ambushing strategy. Hunter ticks typically stay in their shelter until they start host-seeking. Then they actively crawl to and onto their hosts. Most soft ticks and also a number of nidicolous hard ticks belong to this group, but also the hard ticks *R. sanguineus* and many *Hyalomma* species including *H. marginatum*. Ambushing ticks, in contrast, seek an exposed position like grass or shrubs and wait there for prolonged periods in order to cling to a passing host. Once on the host's skin, ticks begin to crawl in search of a place to feed.

Commonly, ticks attach to human skin along pant or sock lines or other tight locations that are warm and humid.

Ticks can be differentiated according to their host preferences:

- "One host ticks": developing stages and adults feed on the same host individual, e.g. *Rhipicephalus microplus*.
- "Two hosts ticks": larvae and nymphs feed on the same host, adults feed on another host, e.g. *H. marginatum*.
- "Three hosts ticks": larvae, nymphs and adults feed on three different host individuals, e.g. *I. ricinus, D. reticulatus*.

5.6.5.13.2 Dossier requirements

Dossier requirements are stated in the Introduction (see section 5.6.5.1.3).

In order to eliminate the risk of disease transmission to human volunteers in field settings, field trials with repellents against ticks are not required for authorisation of products applied on humans or clothing (for details see Introduction, section 5.6.5.1.3.4.3). In the case of products applied to domestic animals, field trials can be less stressful for the animals and should therefore be accepted instead of simulated-use trials.

For authorisation of products applied on human or animal skin efficacy must be proven with the recommended dose and according to the claimed mode of application given on the label and SPC. The dose used in the efficacy studies should be covered by human health risk assessment.

5.6.5.13.2.1 Test species

Products with the claim "against ticks" applied for the use on human skin or for the use as surface or spatial treatment has to be tested either on *I. ricinus* or on *I. scapularis.* Note that for some active substances, efficacy may be different and then the use of the chosen species should be justified, see Appendix 18: Büchel K., et al.. For a species-specific claim, testing against the claimed species is required. The efficacy of products applied on humans should be proven against adults and nymphs. Depending on which developmental stage parasitises the host, the biological relevant developmental stage must be tested (e.g. nymphs and adults for *Ixodes ricinus* and *I. scapularis;* adults for *Dermacentor reticulatus, D. marginatus* and *Hyalomma marginatum*). Therefore, the applicant should provide information and justification on the developmental stage of the ticks used in the efficacy test. For a general claim against ticks, both *Ixodes* adults and nymphs have to be tested, otherwise, the tested developmental stage needs to be stated in the SPC and on the label claim. If CPTs with nymphs and adults are different, the shortest CPT should be stated on the SPC and label.

For a general claim for the repellence of ticks on dogs or products intended for surface treatment when dogs are present, at least two different tick species parasitising dogs should be tested (*R. sanguineus* and either *I. ricinus* or a European *Dermacentor* species). For species specific claims, just the target species has to be tested. The repellent effect for products applied on domestic animals should be demonstrated for each tick species and each target animal species, i.e. dog, horse, etc. claimed. Dogs should be used as a substitute for cats,

however, as testing with cats can imply certain animal welfare issues. The efficacy of products used on domestic animals should be proven against adult ticks because testing with nymphs is not easily feasible even on short animal fur.

There are further *Rhipicephalus* species difficult to differentiate from *R. sanguineus*. Therefore, the species of the test organism should be well-defined, and the origin of the organisms specified.

When use in tropical areas is claimed, *Hyalomma marginatum* or *Amblyomma variegatum* should also be tested. *H. marginatum* behaves differently than *I. ricinus* and *I. scapularis*, since it actively seeks the host to feed on and moves quickly on the ground.

Due to the specificity of certain active substances, for products based on an active substance with a species-specific mode of action, only effects against tick species that have been tested under simulated-use and/or field conditions, depending on the type of claim, should be claimed on the product label and in the SPC.

Table 39: Overview of the required test species depending on the claim

Type of product	Claim	Test species	Developmental stage
Topical repellents on human skin, Surface repellents, Spatial repellents	General claim against ticks	*Ixodes ricinus* or, *Ixodes scapularis*	Nymphs and adults
	Species-specific or developmental-specific claim	Claimed species	Claimed developmental stage
	Use in tropical areas	*Ixodes ricinus,* or *Ixodes scapularis* and either *Hyalomma marginatum,* or *Amblyomma variegatum*	*Ixodes* species: Nymphs and adults Others: Adults
Topical repellents on domestic animals, Surface repellents in the area of domestic animals	General claim against ticks	*Ixodes ricinus* and either *Rhipicephalus sanguineus,* or European *Dermacentor* species or *Hyalomma* species	Adults
	Species-specific claims	Claimed species	Adults
Attractants	Only species-specific claims possible	Claimed species	Claimed developmental stage

5.6.5.13.2.2 Requirements per type of claim and test methods

A laboratory test is not required for product authorisation but can be used to determine one or several effective concentrations under laboratory conditions and to see whether the product sufficiently repels/attracts under laboratory conditions. For active substance approval only, a laboratory test is sufficient. However, the laboratory test can be waived for active substance approval when a suitable simulated-use test is provided.

5.6.5.13.2.2.1 Products intended for use as topical repellents for human skin

The efficacy of repellent products targeting the use on human skin should be proven in a simulated-use test according to the instructions for use (description see 5.6.5.13.2.2.1.2).

In order to eliminate the risk of disease transmission to human volunteers in field settings, field trials with repellents against ticks are not required for authorisation of products applied on human skin (for details see Introduction, chapter 5.6.5.1.3.3.3). Pre-existing studies may be submitted as additional information. If field trials are conducted, they must take place in an area with an appropriate tick density and at a time when the relevant tick species are abundant. These tests should preferably take place in Europe or other relevant regions according to the claims (e.g. tropical regions). Where testing in Europe is not possible, the conditions and tick species must be confirmed, and their relevance justified. Time of day at

which volunteers are treated and at which exposure started and ended should be reported along with the weather conditions (temperature, humidity, precipitation, wind speed, light intensity, cloudiness). At least 10 volunteers (preferably an equal number of males and females; age: 18–65 years) should be included in the field trial. To avoid tick bites on volunteers negative controls are not necessary, because field trials can only be used as supportive data. If field trials are conducted, care must be taken to avoid tick bites.

The repellent products used for the test should be identical to the product to be marketed.

CPT values must be determined in a simulated-use test. As field studies are not assessed as key studies, data from the field studies cannot be used to determine CPT values.

5.6.5.13.2.2.1.1 Laboratory test

Different set-ups are recommended for testing repellent products targeting the use on human skin, see Appendix 18. Only one appropriate set-up is required per use.

5.6.5.13.2.2.1.2 Simulated-use test

Volunteers: Efficacy testing should involve at least 10 different volunteers (preferably volunteers with different hairiness of the arms, different genders; age: 18-65 years). For CPT calculation valid data of at least 10 different volunteers must be used. Volunteers need to be fully informed about the aim, the procedure and the expected duration of a study. The study should be carried out in compliance with the national ethics regulation. They also need to be informed about potential side effects such as allergic skin reactions caused by the product. Every side effect observed during the test should be mentioned in the test report. Their participation is voluntary and can be recalled at any time before and during the study, see Appendix 18: OPPTS 810.3700. 12 hours before and during testing, volunteers should avoid nicotine, alcohol, fragrances (perfumes, body lotions, soap, etc.) and repellent products. Prior to the application of the repellent, the skin is washed with fragrance-free soap and rinsed with water.

Test design: The repellent product is applied to human forearms from approximately 5 cm above the wrist to the elbow. As a negative control, an untreated arm of the same test person will be tested. This also serves to pre-screen ticks for sufficient crawling activity. The criteria of test time and distance in the control should correspond to the test run with the repellent product. On the control arm, a line is drawn approximately 5 cm above the wrist to mark the beginning of the crossing zone. To mark the release point of the ticks a line is drawn at a distance of 3 cm below the crossing zone, see Figure 15. To mark the end of the crossing zone, a third line is drawn 3 cm above the line that marks the beginning of the crossing zone. In order to be considered as sufficiently locomotive, a tick needs to cross the crossing zone. On the treated arm, the same lines as above are drawn. Ticks showing sufficient crawling activity are used for the test on the treated arm immediately after the control test.

Test animals must be active for host searching behaviour, which is after a starvation period of a minimum of 6 months (for *Ixodes*, for other species a shorter starvation period of 3 months is possible) after the last blood meal. Ticks must be pathogen-free. The arms are inverted to promote upward movement since most ticks are negatively geotropic. Ticks are placed on the untreated release point either on the bottom or the upper side of the arm 3 cm below the test area with forceps or a brush. The side used must be documented in the study report. The same side of the arm must be used during the entire study to get comparable data. While *I. ricinus* moves upwards by themselves, *I. scapularis* needs to be gently 'guided' upwards using a paintbrush (see Appendix 18: Carroll S.P.). Each tick that has crossed into the marked 3 cm zone and crossed the 3 cm zone (tick species like *I. ricinus*) or stayed there for 1 min (tick species like *I. scapularis,* which is a species with low locomotive activity) is considered as sufficiently locomotive and can be directly used as a test tick on the treated arm.

Exposure periods should take place every 30 or 60 minutes with the first being 30 or 60 minutes after the product application. Each tick should be observed for a maximum of 3 minutes. 5 or 10 ticks should be tested within each 30 or 60 minutes intervals, respectively, by each volunteer, giving a total of 10 ticks per hour and volunteer. If two species are tested in one trial the same criteria are applicable, i.e. 10 ticks per hour and volunteers must be tested per species. For testing both nymphs and adults for a general claim against ticks 5 nymphs + 5 adults (= 10 ticks) per hour and volunteers must be tested. For testing only one developmental stage 10 ticks (either 10 nymphs or 10 adults) per hour and volunteers must be tested.

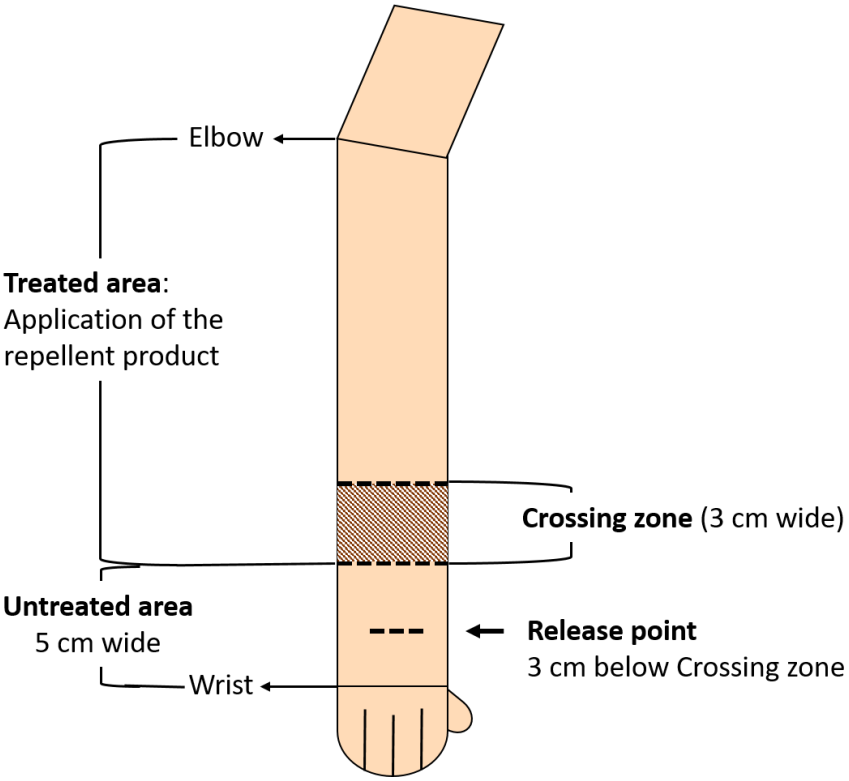

Figure 15: Upper side of a human test arm in a simulated-use test to show repellency against ticks. Dotted lines (release point, start and end of the 3 cm crossing zone) mark the lines that are drawn on both the control and the test arm before the test.

The following criteria for repellence should be used:

A tick is not repelled when it, within the 3 minutes observation time, either

- Crawls onto the treated area and remains there for 1 min, or
- Enters the treated area and further crawls across the 3 cm crossing zone within less than 1 min after entering the treated area.

A tick is repelled when it, within the 3 min observation time, either

- does not crawl into the treated area, i.e. stays on the untreated area or drops off from the untreated area of the treated arm after contacting the start line of the crossing zone (repellent border), or
- crosses the start line of the crossing zone (repellent border), but turns back into the untreated area within less than 1 min after entering the treated area, or
- crosses the start line of the crossing zone but drops off from the treated area within less than 1 min after entering the treated area.

A tick that does not move at all on the test arm, even after moving on the control arm, has to be excluded from the test, and another tick should be used instead. Every tick should only be used once for a test to avoid habituation effects. When a tick starts to bite, this tick should be removed directly from the skin. When a tick starts to bite hereby on the treated area this tick is considered as non-repelled. Biting can be prevented by constant observation.

Efficacy assessment: While testing for repellence, an endpoint for the failure of repellence for subjects treated with the recommended dose should be selected. Efficacy failure in a test to determine CPT is the time from the application of a repellent until efficacy failure by a confirmed event (definition see Introduction, section 5.6.5.1.5). Repellent effectiveness should be based on the median or mean CPT (CPT calculation see Introduction, section 5.6.5.1.5).

The claimed CPT for a general claim should correspond to the shortest CPT (mean or median) among all tested species and should be stated on the label and SPC. For specific claims, the mean or median for each species respectively may be stated separately on the label and SPC.

If different developmental stages have been tested, the shortest CPT (mean or median) should be indicated on the label and SPC.

Proof of non-insecticidal efficacy, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8): The same test setup as described above can be used with a defined number of ticks (at least 50 individuals, with 5 individuals per volunteer). Ticks are placed in the treated area on the line that marks the end of the crossing zone. The arms are inverted to promote upward movement since most ticks are negatively geotropic. To simulate a natural situation, ticks should be removed after one minute if they do not leave the treated area by themselves. Afterwards the ticks should be kept separately in the laboratory and mortality should be recorded 24 hours after exposure. This test should be conducted within the 30 minutes directly after the product application and before the first exposure of ticks for repellency testing.

5.6.5.13.2.2.2 Repellents applied on clothing both for humans or animals

For product authorisation purposes the efficacy of repellent products applied on clothing for humans should be proven in a simulated-use test according to the instructions for use (description see 5.6.5.13.2.2.2.1).

CPT values must be determined in a simulated-use test. As field studies are not assessed as key studies, data from the field studies cannot be used to determine CPT values.

In order to eliminate the risk of disease transmission to human volunteers in field settings, field trials with repellents against ticks are not required for authorisation of products used on humans (for details see Introduction, section 5.6.5.1.3.4.3). Pre-existing studies may be submitted as additional information. If field trials are conducted, they must take place in an area with an appropriate tick density and at a time when the relevant tick species are abundant. These tests should preferably take place in Europe or other relevant regions according to the claims (e.g. tropical regions). Where testing in Europe is not possible the conditions and tick species must be confirmed, and their relevance justified. Time of day at which volunteers are treated and at which exposure started and ended should be reported along with the weather conditions (temperature, humidity, precipitation, wind speed, light intensity, cloudiness). At least 10 volunteers (preferably volunteers with different hairiness of the arms, different genders; age: 18–65 years) should be included in the field trial. To avoid tick bites on human volunteers negative controls are not necessary, because field trials can only be used as supportive data. If field trials are conducted, care must be taken to avoid tick bites.

For product authorisation purposes the efficacy of repellent products applied on clothing for animals should be proven in:

- a simulated-use test according to the instructions for use, or
- a field trial according to the instructions for use.

The repellents used for the test should be identical to the product to be marketed.

Proposed claims regarding the protection of a specific type of fabric must be simulated, i.e. the same type of fabric has to be used in the simulated-use test.

Proposed claims regarding the performance of the product need to be simulated in the study (for details see Introduction, section 5.6.5.1.3.5).

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or repellent product (temperature, humidity, photoperiod, ventilation).

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for details see Introduction, section 5.6.5.1.4.1.3).

The efficacy data should be relevant to prove the submitted claims. Therefore, the efficacy of biocidal products that are limited to a body area, e.g. collars must be proven for the whole test individual that should be protected. If only specific body areas intend to be protected, e.g. ears, udders, etc. then the efficacy for the claimed area must be proven.

For specific claims (prevention of bites through/prevention of bites next to the treated clothes), relevant tests have to be submitted.

5.6.5.13.2.2.2.1 Simulated-use test

The efficacy of products intended for use on clothes or fabrics for humans should be proven in a similar test as described in section 5.6.5.13.2.2.1.2 by covering the test arm with a treated cloth and the control arm with an untreated cloth. The fabric used has to be representative to the fabrics commercially used, i.e. thick uniform cotton if the field of use is military clothing, etc. The ticks should be released in an untreated area, e.g. fabric tape on the textile, allowing the tick a barrier-free transition to clothing.

For efficacy assessment and determination of non-insecticidal efficacy please see 5.6.5.13.2.2.1.2.

5.6.5.13.2.2.3 Repellent products intended for use as spatial treatment

For product authorisation purposes the efficacy of repellent products intended for use as spatial treatment should be proven in:

- a simulated-use test according to the instructions for use, or
- a field trial according to the instructions for use.

These kinds of products are not sought for the application to humans or animals. Therefore, field studies can be conducted without human or animal probands and the risk of vector transmitted diseases can be neglected.

The repellents used for the test should be identical to the product to be marketed.

Replicates: A minimum of 5 independent replicates (each treatment and negative control) should be performed. The pre-treatment counts of ticks or control plots can serve as negative controls.

For a general claim "spatial repellents" the repellent effect must be proven. For a specific claim "dispelling" the dispelling effect must be proven.

For residual efficacy, ticks are exposed to the product at several time intervals after application (including the end of the claimed period).

The room size stated in the SPC and on the label must be used in the efficacy trial. For the extrapolation to larger room sizes than proven in the efficacy test a justification is necessary.

If outdoor use is claimed, the test should be performed outdoor and the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for detailed information regarding the requirements see Introduction, section 5.6.5.1.4.1.3).

Proof of non-insecticidal efficacy, if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8) have to be examined at the end of the trial.

5.6.5.13.2.2.4 Repellent products intended for use as surface treatment

For product authorisation purposes the efficacy of repellent products should be proven in:

- a simulated-use test according to the instructions for use, or
- a field trial according to the instructions for use and additionally a laboratory trial testing the required different surfaces.

These kinds of products are not sought for the application to humans or animals. Therefore, field studies can be conducted without human or animal probands and the risk of vector transmitted diseases can be neglected.

Products applied onto surfaces may act either by evaporation or on the surface itself.

For products applied on surfaces, two porous and one non-porous surface should be used, e.g. ceramic tile, plywood, painted plywood, stainless steel, concrete, for a general claim as "surface treatment". The efficacy of each surface should be proven in a separate test, i.e. three tests for three different surfaces. For authorisation of a product to be used on a specific type of surface the efficacy for only this specific surface should be assessed.

Replicates: A minimum of 5 independent replicates should be performed (each treatment and negative control).

For residual efficacy, ticks are exposed to the product at several time intervals after application (including the end of the claimed period).

If outdoor use is claimed, the treated surface/substrate or repellent product and control surfaces/substrates have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for detailed information regarding the requirements see Introduction, section 5.6.5.1.4.1.3).

Proposed claims regarding the performance of the product should be simulated in the study. For example, for the claim "unaffected by cleaning/vacuuming", the surface should be repeatedly cleaned during the trial (for details see Introduction, section 5.6.5.1.3).

Proof of non-insecticidal efficacy, if it cannot be waived, have to be examined in a simulated-use test (for details see Introduction, section 5.6.5.1.3.8).

5.6.5.13.2.2.5 Repellents applied on animals

For product authorisation purposes the efficacy of repellent products applied on animals should be proven in:

- a simulated-use test according to the instructions for use (test design example see 5.6.5.13.2.2.5.1), or
- a field trial according to the instructions for use.

Due to practical reasons, tests on animals should be performed with adult ticks, as nymphs might be more difficult to handle on animal fur and as the adult stage is the most common stage found on companion animals like cats or dogs.

Topical repellents for animals should be tested against the targeted tick species and on the animal, e.g. dog, horse, etc. that shall be protected. Dogs should be used as a substitute for cats, as testing with cats can imply certain animal welfare issues.

The repellents used for the test should be identical to the product to be marketed.

Test animals: It is recommended to include at least 10 animals per treatment/control group of different breed and sex, i.e. for testing on entire animals at least 10 treated and 10 control individuals are necessary since repellence/attractiveness to ticks varies considerably between animal individuals.

Depending on the animal species, several factors, e.g. hair length, the thickness of the coat, self-grooming, etc. might impact the efficacy. These factors should be taken into account in the demonstration of the efficacy. The two groups should be kept separately, to avoid contact of the control animals with the treated fur.

The product should be applied according to the claim. Claimed application rates should take into account the type and weight of the animals. The dose used in the efficacy studies should be covered by risk assessment.

It is necessary that animals come from suitable, e.g. non-smoking households. Every side effect observed during the test should be mentioned in the test report. 24 hours before and during testing, animals should not be treated with fragrance (perfumes, soap, etc.). The test animal should not be protected by any previous repellent and/or insecticidal residuals caused by previous treatment. Concerning endo-antiparasitic agents this is acceptable, but details (product, dose, application rate, application method, date of the last treatment, and residual efficacy) must be given in the report and the controls and the treated groups must have the same status. A safety margin for externally applied anthelmintics is 4 weeks after the end of the last application. The history of the treated and the control groups should be comparable. The status of the animals should be specified and only animals for which an insecticidal effect can be excluded should be included in the study. Due to feasibility and cost-effectiveness, it would be possible to use 10 animals one week (5 each in the treatment and control groups) and then use the same individuals again in the second week, but swap groups. Each animal would then act as its own control. However, any residual activity of the product has to be excluded.

The investigator is reminded that the validity of the results is directly related to the degree of variability in the test. Increasing the number of test animals could increase the reliability of the test results.

Proposed claims regarding the performance of the product should be simulated in the study. For the claim "unaffected by washing", the label and the SPC must indicate how often the animal can be washed without reducing the efficacy of the biocidal product. In the efficacy test, the test individuals should be repeatedly washed with a non-insecticidal, fragrance-free shampoo during the trial according to the number of wash cycles indicated. If a product claim is to protect the entire animal, this should be demonstrated (notably for collars).

5.6.5.13.2.2.5.1 Simulated-use test

The candidate repellent is applied according to the instructions for use. If the product is acting at very close range, it may be possible to make the evaluation by treating parts of the host, like one treated side of the neck compared with the equivalent area on the other, untreated, side of the neck. The advantage of such a setup is the neutralisation of individual host differences in tick-attraction. The control body part should resemble the treated body part (e.g. front-half vs back-half or left side vs right side of the animal). In case of a restricted application, e.g. collar, a spot-on product with a claim of entire body protection or for products acting in a wider range over the entire body the test should permit to validate the efficacy of this kind of application by testing the entire animal. In this case untreated animals must be used as a control. The negative controls serve to pre-screen ticks for sufficient locomotive activity.

The attractiveness of the animal should be validated before the trial. Animal's attractiveness can be measured by exposing the untreated skin to ticks.

The detailed measurements will depend on the size of the animal, and the following dimensions adapted from the simulated-use test on humans should be used only as guidance. Other test designs than the following examples can be accepted if the protocol is scientifically valid.

Test design: On the control leg as well as on the treated leg, a 3 cm width crossing zone is marked (e.g. by drawing or shaving a line) approximately 5 cm above the carpal joint. The release point of the ticks is marked by a line at a distance of 3 cm below the crossing zone. In order to be considered as sufficiently locomotive, a tick needs to cross the crossing zone.

Alternatively, products can be applied according to the label claim and SPC on one side (= treated side) of the test animal, e.g. the lateral area of the thorax. The other side of the test animal should be used as negative control to evaluate sufficiently locomotive ticks. During the observation the animals are allowed to stand, sit or lay down. The ticks can be placed on the fur of the treated side on an untreated sleeve. The ticks must be released on an untreated area of at least 1 cm width. In order to be considered as sufficiently locomotive, a tick needs to walk on the untreated side into the fur to the skin within 3 minutes observation time. Biting can be prevented by constant observation.

Ticks must be active for host searching behaviour, which is after a starvation period of a minimum of 6 month (for *Ixodes*, for other species a shorter starvation period of 3 months is possible) after the last blood meal and must be pathogen-free. Ticks are placed on the untreated release point 3 cm below the crossing zone with a forceps or brush. While *I. ricinus* moves upwards by themselves, *I. scapularis* needs to be gently 'guided' upwards using a paintbrush (see Appendix 18: Carroll S.P.). Each tick that has crossed into the marked 3 cm-zone and crossed the 3 cm-zone (high locomotive tick species like *I. ricinus*) or stayed there for 1 min (low locomotive tick species like *I. scapularis*) is considered as sufficiently locomotive and can be directly used as test tick on the treated leg.

Alternatively, a tick that has walked on the control side into the fur to the skin within 3 minutes observation time is considered as sufficiently locomotive and can be directly used as a test tick on the treated other side of the thorax.

Exposure periods should take place every 30 or 60 minutes with the first being 30 or 60 minutes after the product application. Each tick should be observed for a maximum of 3 minutes. 5 or 10 ticks should be tested within each 30 or 60 min intervals, respectively, by each test animal, giving a total of 10 ticks per hour and test animal. If two species are being tested in one trial the same criteria are applicable, i.e. 10 ticks per hour and test animal must be tested per species.

The following criteria for repellence can be used:

A tick is not repelled when it, within the 3 minutes observation time, either

- crawls onto the treated area and remains there for 1 minute, or
- enters the treated area and further crawls across the 3 cm crossing zone within less than 1 minute after entering the treated area, or
- crawls onto the treated area from the fur to the skin.

A tick is repelled when it, within the 3 minutes observation time, either

- does not crawl into the treated area, i.e. for the test design with marked areas the tick stays on the untreated area or drops off from the untreated area of the treated area after contacting the repellent border (start line of the crossing zone), or
- crosses the start line of the crossing zone (repellent border), but turns back into the untreated area within less than 1 minute after entering the treated area, or
- crosses the start line of the crossing zone but drops off from the treated area within less than 1 minute after entering the treated area.

A tick that does not move at all on the test area, even after moving on the untreated negative control area, has to be excluded from the test, and another tick should be used instead. Every tick should only be used once for a test to avoid habituation effects.

For efficacy assessment and proof of non-insecticidal efficacy, if it cannot be waived, please see 5.6.5.13.2.2.1.2.

The investigator is reminded that the validity of the results is directly related to the degree of variability in the test. Increasing number of test animals could increase the reliability of the test results.

Authorisation of products applied with a dose independent of animal size can be granted up to the bodyweight of the heaviest test animal.

The claimed CPT for a general claim should correspond to the shortest CPT (median or mean) among all tested species and should be stated on the label and SPC. For specific claims the mean or median for each species respectively may be stated separately on the label and SPC.

Environmental conditions must be specified for the test itself (temperature, humidity, light intensity).

5.6.5.13.2.2.6 Attractants without PT18 active substances

For product authorisation purposes the efficacy of attractants in traps should be proven in:

- a simulated-use test according to the instructions for use, or
- a field trial according to the instructions for use.

Any attractant should be tested against a negative control.

The attractants, e.g. the traps, used for the test should be identical to the product to be marketed (for details see Introduction, section 5.6.5.1.3.7). Any bycatch of non-target insects has to be recorded and identified at least to order, preferably to the family level.

Environmental conditions must be specified for the test itself and during the storage of the treated substrates/surfaces or attractant product (temperature, humidity, photoperiod, ventilation).

If outdoor use is claimed, the attractant product and control have to be kept outside to ensure adequate weathering and the outdoor conditions must be specified (for detailed information regarding the requirements see Introduction, section 5.6.5.1.4.1.3.).

Replicates: A minimum of 5 independent replicates should be performed (each treatment and negative control).

In field trials the tick population can be determined by flagging of the vegetation.

5.6.5.13.2.2.7 Attractants in PT18 bait products

The efficacy evaluation of such products should be done on the basis of the requirements for PT18 products (see PT18 Chapter: Insecticides, acaricides and products to control other arthropods).

5.6.5.13.3 Assessment of authorisation

5.6.5.13.3.1 Norms and criteria

According to the BPR, a biocidal product may only be authorised if it "*possesses a sufficient level of efficacy*". This is implemented for ticks in the following way:

For repellent products non-insecticidal efficacy has to be proven in a simulated-use test if it cannot be waived (for details see Introduction, section 5.6.5.1.3.8).

Products intended for use as repellent for human and animal skin, clothing

The required results for simulated-use tests and field trials are:

- during the claimed protection period complete protection should be proven expressed as mean or median CPT (for more details see Introduction, section 5.6.5.1.5)
- CPT should correspond to the shortest CPT (mean or median) among the tested species and developmental stages

Products intended for use as repellent as spatial or surface treatment

The required results for laboratory tests, simulated-use tests and field trials are:

- during the claimed protection period ≥ 80% repellency;
- Protection period should correspond to the shortest period among the tested species.

Products intended for use as attractants without PT18 active substance

The required results for the different trials are:

- at least a ratio of 4:1 of test individuals trapped in the trap with attractant compared to the control trap within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

Laboratory test or simulated-use test:

- ≥ 80% trapped test individuals compared to the negative control within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

Field trial:

- ≥ 80% trapped test individuals compared to the negative control within the test period (or according to the claim), from the beginning and until the end of the claimed efficacy period.

5.6.5.14 Wasps

NOTE to the reader:

This section of the guidance contains limited information concerning repellents against wasps included previously in chapter 5.6.4 and will be updated in the future in light of the experience gained. The applicant should provide a testing proposal which needs to be agreed upon by the respective CA in advance on a case-by-case basis.

5.6.5.14.1 Test species

A product for use against wasps should be tested on colonies and/or workers of *Vespula* spp. or *Dolichovespula* spp.

5.6.5.14.2 Requirements per type of claim and test methods

For products with a repellent or attracting effect against wasps no agreed protocols are available. The tests should be designed to mimic the practical use situation. The study results should provide a clear picture of the efficacy of the product. Methods should be described well. The submitted data from studies are checked for completeness, based on the applied dose per treated area. It is also checked whether the duration of exposure is sufficient. If the formulation alone i.e. without the carrier, e.g. a product with a tissue as carrier, has been tested, data on release from the carrier are also required.

Products intended for repelling wasps

- simulated-use or field trials.

5.6.5.14.3 Assessment of authorisation

5.6.5.14.3.1 Norms and criteria

Products intended for repelling wasps

- required results in a simulated-use or field trial:
  - a simulated-use test showing repellence;
  - depending on the claim field trial showing repellence.

5.6.6 PT20 Other vertebrates

Please refer to the General sections 1-3 of this guidance and the TNsG.

5.7 Other biocidal products (Main group 4)

5.7.1 PT21 Antifouling products

5.7.1.1 General Introduction

This section deals with the methodology for the evaluation of efficacy tests for antifouling products that is applicable for the authorisation of products under the EU Biocidal Products Regulation (BPR, Regulation (EU) 528/2012).

5.7.1.1.1 Introduction

This chapter describes the nature and extent of data which should be available to support the label claims for biocidal products within Product Type 21 - Antifouling Products. These are defined in the BPR as "Products used to control the growth and settlement of fouling organisms (microbes and higher forms of plant or animal species) on vessels, aquaculture equipment, or other structures used in water".

5.7.1.1.2 Types of Coating

The antifouling products currently available can be categorised into the following broad coating types:

- Soluble matrix
- Insoluble matrix
- Self polishing

The categorisation of coating types outlined above is general. It should be noted that some antifouling products do not necessarily rely on one single coating technology and combinations of different technologies have been developed by antifouling formulators to suit customer specifications and environmental requirements. A description of the main coating types can be found in Appendix 20.

It should be noted that the protection periods described in the appendix for each coating type are typical life times that may be achieved by using products within these very broad groups. The efficacy of an antifouling coating will heavily depend upon use, for instance a vessel's operational pattern (such as dry-docking interval, sailing speed, and idle times as well as the temperature, fouling intensity, and other environmental characteristics where the vessel is trading). It also depends on the extent to which the antifouling paint specification has been tailored to meet these specific conditions. Surface preparation, primers, quality of work, dry film thickness, etc. may also affect the quality and/or duration of the protection.

5.7.1.1.3 Mode of Action

Antifouling products form paint films that act as release vehicles for the active substance(s) contained in the paints. The active substance(s) will be released over the specified lifetime of the products, creating a microlayer of biocide rich water at the paint surface. Here, in this water microlayer, the concentration should be sufficient to deter the settlement and/or growth

of fouling organisms. A more detailed description of the respective modes of action and physical characteristics of the various coating types are outlined in Appendix 20 of this document.

5.7.1.1.4 Categorisation of antifouling products

Antifouling paints are made available for different use types. Typically they are prescribed for yachts, commercial vessels (such as bulk carriers, tankers, container ships, car carriers, passenger ships, etc.), and aquaculture.

The three broad categories of products (in Appendix 20) can be defined by the way in which the products control the release the active substance(s). Given the fact that a single active substance may not have a sufficiently broad spectrum activity to control the wide range of fouling organisms, antifouling products often contain more than one active substance.

5.7.1.1.5 Spectrum of activity

Target organisms belong to very different taxonomic groups. There are many organisms that can live within a fouling community, but only a few cause severe fouling problems. Which organisms will present a problem depends on the local conditions and the operation of the individual vessel. For example, typical target organisms in European waters may include, but are not limited to, various species of the following genus: *Pseudomonas* (light slime), *Amphora* (dense slime), *Ulva* (macro-algae), and *Semibalanus* (animals).

Fouling organisms and growth rates differ between tropical and temperate regions. The fouling intensity and the species that dominate a fouling community may vary locally and seasonally. While it is not normally feasible to claim efficacy against specific target organisms, applicants may choose to supplement their label claim that the product is an 'antifouling product' with an indication as to whether the product will be effective against one or more of the following fouling groups:

- Slime
- Weed (macro-algae)
- Animals

5.7.1.1.6 Dossier requirements

The following aspects are required for the efficacy evaluation of antifouling products:

1. The label claims and instructions for use including the technical data sheet
2. Efficacy data on the product

5.7.1.1.7 Label claims

For each product a set of label claims should be provided as part of the dossier submitted. Claims for the activity of the product include those made on a technical data sheet or other associated documentation, as well as those on the label itself. To simplify the text, only the term 'label claim' will be used below.

In general the claim for antifouling products can be rather unspecific, for instance 'antifouling product for professional application'. The label should also indicate to which fouling groups (see 5.7.1.1.5) the product is effective and whether it can be used in marine or fresh water.

The label claim for products used in areas other than on vessels, such as products used for aquaculture, in the inlet and outflow pipes of cooling systems, or for other "non-vessel" uses should be more precise, and clearly describe purposes for which the product can be used.

According to Article 69(2)(f) of the BPR the label must clearly and indelibly show the uses for which a biocidal product is authorised.

5.7.1.1.7.1 Areas Of Use

The product label, technical data sheet or other associated documentation should contain information on the main use categories for the product, for example use on vessels and larger boats, yachts, stationary installations, or aquaculture equipment, etc. This will normally also include information on whether the product is intended (primarily or exclusively) for use in either marine or fresh water.

As the fouling challenge is more severe under static conditions, installations and recreational boats (which are normally tied up in marinas) will foul more quickly than commercial vessels that spend most of their time in motion. Therefore, if a product is intended specifically for static or recreational use, this should be specified in the label claims.

(For human risk assessment purposes, it is important that a label claim specifies if a product is intended for amateur use or if is for application by professionals only.)

5.7.1.1.7.2 Application method/dose rate

Antifouling coatings may be applied using methods such as airless and conventional spray, brush and roller, or dipping and immersion (aquaculture). The specified total dry film thickness will vary depending on the intended dry-docking interval, activity of the vessel (such as sailing speed and idle times), and on the temperature, fouling intensity, and other environmental characteristics where the vessel is operating. Furthermore, larger vessels will normally have different antifouling products and different paint film thicknesses specified for different parts of the underwater hull depending on, for instance, water flow and light conditions. Some areas, such as those with less frequent maintenance intervals than those for the rest of the underwater hull, and those with strong water throughput (e.g. inside thrusters) may require higher film thicknesses to minimize the risk of transmigration of non-indigenous species in these areas.

It is important to note that the paint thickness does not affect the efficacy of a product, which will control fouling regardless of the thickness of the paint applied. Instead, the film thickness will define the in-service life of the product.

For antifouling paints there is no direct relationship between the applied dose (paint film thickness applied) and the efficacy of the product (unlike agrochemicals, for example, where applying more pesticide increases the concentration of the pesticide and therefore the magnitude of the controlling effect on the pest).

Recommended dry film thicknesses are given to ensure that enough paint is applied to the vessel to avoid the coating being 'polished through' during service, exposing the underlying anticorrosive paint which will be susceptible to fouling. When paint is applied by spray, more than one coat of paint is normally applied to protect against possible application defects, such as 'pin holing', where small areas of the anticorrosive are left exposed.

As the three major types of antifouling coatings (Appendix 20) vary in their ability to maintain a sufficient release of active(s), this is reflected in their different typical lifetimes.

5.7.1.1.8 Efficacy tests

5.7.1.1.8.1 Laboratory tests (including in-vitro screening tests)

Laboratory tests are typically conducted on a single active substance and with a limited number of test organisms, and may provide information about the specific action of a substance against a known fouling species. It is acknowledged that model target organisms may be used in these tests as well as those that may successfully be cultivated in a laboratory (e.g. juvenile barnacles). Consideration should be given to the use of species known to be critical fouling species.

Laboratory tests are routinely used to demonstrate efficacy of an individual active substance, often at a very early stage during research in order to screen new active substances.

Laboratory testing of individual paints is not undertaken as it is not considered to be a realistic evaluation of the product. Field testing is routinely undertaken instead (described below).

5.7.1.1.8.2 Simulated field trials (static raft testing)

These may be studies that are conducted with the candidate product or with the active substance(s) incorporated into a model coating type. Such tests involve the immersion of panels treated with the test coating on static rafts for a period of months or years at an appropriate location. For aquaculture products this could be nets or (sections of) cages treated with the test product and immersed at an appropriate site.

Efficacy data on antifouling coatings should normally be generated by testing over at least six months of peak fouling activity. As far as is practical the test location(s) should be representative of the intended uses of the product. When testing in locations with seasonal

variation in fouling challenge, the test period should cover the full fouling season. The length of a season will vary depending on the location of the test site. When choosing the test location(s), factors such as shelter (from strong waves and ship traffic) and access have to be balanced against water exchange conditions and other characteristics determining whether the water at a site is representative for the end use conditions.

Since raft testing is carried out in natural environments, the same product may perform differently at the same site in different years. This variability in fouling intensity, and thus the test results, is due to weather conditions, availability of nutrients, and other uncontrollable factors that may affect the type and extent of fouling and its rate of settlement and growth. Therefore, a negative control (a surface which has no antifouling effect) should be included in all tests, which will indicate the degree of fouling that would be present under static conditions if the tested coatings were totally ineffective. A reference coating of proven or known efficacy (a positive control) may also be used. The absolute amount of fouling present on a test coating may not be reproducible at the same site from year to year.

Efficacy studies include regular assessments of fouling throughout the period. These assessments usually describe the major types of fouling (e.g. slime, algae and other weeds, and barnacles or other fouling organisms), but describing these as to genus and species is unnecessary. As sharp edges on test panels may be difficult to protect, fouling that is not growing on the front of panels (i.e. attached along the edges) should be disregarded.

The presentation of data should include the assessment method (the rating/scoring for the test panels and how these are interpreted), together with photographs and/or diagrams of the test panels.

5.7.1.1.8.3 Field trials/In-service monitoring

Since field trials involve long-term exposure to practical conditions, they can be regarded as in-service tests. Field trials permit antifouling products to be tested under similar operating conditions and stresses as those encountered when the antifouling product are in service. Possible examples of these tests include:

- Panel tests where coated panels have been attached to a vessel during parts of or during a complete dry-docking interval
- Patch tests where vessels have been painted with the test coating as a strip or patch on the hull
- In-service monitoring of aquaculture nets, cages, etc.

Any field data generated in support of an application should be conducted on the candidate product or representative products that closely resemble the fully formulated commercial product. A robust justification should be provided to support bridging of data from a similar (but not identical) product.

It is recognised that it may not be possible to run concurrent untreated panels or patches during field trials. Therefore information on the performance of the main antifouling coating over the test period should be provided instead. Monitoring reports of the performance of an antifouling product on a fully treated vessel may also be submitted, where these are available. It is also recognised that data generation from field trials may require many years to carry out and are more likely to be available for well-known technologies than for products containing new active substances (or new combinations or concentrations of active substances) or for coating types based on new technologies.

Where field data are not available, the applicant has the option to provide data on other existing formulation(s) where appropriate, and read-across to the current application through scientific reasoned cases and arguments. Such arguments may include:

- The composition of the 'old' (and well documented) and the 'new' antifouling product
- Simulated field trials of the ‘old’ and the ‘new’ antifouling product
- Possible field data on the 'old' antifouling formulations
- Further justification, such as why bridging is appropriate (e.g. in-service monitoring)

It is understood that extensive field data or bridging data may not be available when established biocides have been introduced into products based on new technology or new active substances are being developed. Field trials from different ships have limited value for the purpose of comparing efficacy due to the diversity of operational patterns and trading

routes and the likeliness for unforeseen circumstances or incidents not recorded. This, together with the complexity with respect to application and monitoring and the long exposure times required, explain why in-service tests are normally not available for new antifouling products. However, when data on in-service/field trials are available, these should be submitted as additional information.

However, field data are required at renewal of a product authorisation, as the product will have been on the market for several years by this point. Further guidance on how to perform and assess these data will be developed in the future and incorporated into this guidance.

5.7.1.1.8.4 Replication of efficacy tests

Antifouling paints are normally tested in series during product development, where panels treated with a range of formulations, with only small variations between them, are tested to assess the effects of exposure on other paint properties, as well looking at the efficacy of the formulations.

Since the testing takes place in a natural environment, the variation in fouling propagation and intensity between different years at the same test site will vary. A variable natural environment, the differences in fouling activity between years, and the criteria for establishing efficacy (the general nature of a label claim) make very detailed evaluations unnecessary.

However, to increase the scientific rigour of the evaluation, the results of three replicate plates should be submitted.

It is acknowledged that it is not common practice to test multiple replicates of individual formulations, however panels treated with similar formulations containing the same combination and concentration of active substances may be considered replicates when these are supported by a suitably robust reasoned case explaining the relevance of these formulations to the candidate product. The results from such panels should be submitted, along with details of the formulations used, as well as the reasoned case.

5.7.1.1.9 Standard test methods

5.7.1.1.9.1 Simulated-use test methods

The standard test methods available for the generation of simulated field data through raft testing of antifouling coatings are:

1. Efficacy evaluation of antifouling products. Conduct and reporting of antifouling efficacy evaluation trials. CEPE Antifouling Working Group, June 2012. This methodology has also been adopted by the International Paint and Printing Ink Council – IPPIC and presented at Technical Meeting I 2013 PT 21 efficacy workshop (Appendix 21).
2. American Society of Testing Methods (ASTM) - ASTM D3623 - 78a (2004) Standard Test Method for Testing Antifouling Panels in Shallow Submergence which is linked to ASTM D6990-5(2011) Standard Practice for Evaluating Biofouling Resistance and Physical Performance of Marine Coating Systems.

Reports based on both the above methods should be accepted.

However, it should be noted that the ASTM methods were primarily developed to satisfy the detailed requirements of the US Navy and are not commonly used by the general antifouling industry. The main reasons for this are that they are resource intensive (in terms of the level of detail required in both the materials used as well as the analysis and reporting of the fouling species [including the number and diameter of individual organisms), thereby exceeding the requirements for substantiating a general product label claim (since normally specify only the general types of fouling and their extent are reported for regulatory purposes)] and that they specify relatively dated materials (paints), for which better and more applicable alternatives are available. Notwithstanding, the methods may provide a good basis for biological research.

5.7.1.1.9.2 Field/In-service tests

There are currently no national or international standards that cover field evaluation of antifouling products. Field trials (application on ships) are rarely used to screen formulations and establish the basis for an efficacy claim since they are time consuming and costly and since the results are heavily dependent upon the operations of individual vessels. To the extent field

trials are used, their purpose is normally to determine relative differences in efficacy between already commercial formulations during different use conditions (such as vessel speed, idle times, etc.).

Typically a new antifouling paint represents an incremental improvement or an adaptation to a specific user requirement. Normally, therefore, the experience from similar commercial products will contribute to the confidence the manufacturer has with respect to the efficacy of a new product.

However, at the point of renewal of a product authorisation, a product will have been on the market for several years and field data should be generated to demonstrate the actual performance of the product in use.

5.7.1.1.10 Resistance

Resistance is discussed in the general part of the TNsG on Product Evaluation in Chapter 6. A review of resistance is part of the evaluation at product authorisation. If new information is available which was not reviewed during the approval of active substance, this information should be provided at the time of product authorisation.

In general development of resistance is not to be expected for marine use, as ships are treated with several antifouling paint products containing different active substances. However, this may not be the case for use in fresh water and aquaculture. Reports of development of resistance should always be mentioned.

5.7.1.1.11 Service life

Amateur antifouling products for recreational crafts are normally claimed to last for one yachting season, and are recommended to be retreated annually. Commercial vessels will have extensive tailor made paint specifications depending on their dry-docking interval and operational pattern. Different products and film thicknesses are frequently used at different parts of the vessel due to different light conditions and hydrodynamic forces. In the case where a label claim includes different types of use (e.g. both vessels and static installations), the corresponding protection times may differ.

With respect to the ability of fouling organisms to settle and attach, static conditions are much more favourable than the conditions on vessels that are only idle for relatively short periods at the time. This together with the greatest levels of marine growth occurring in near shore conditions (as described in 2.1), explain why static raft testing is a worst-case test. For recreational craft, however, the use conditions may be very different. Therefore, tests are frequently carried out for the same number of fouling seasons as the recommended use.

It is not obligatory to state on the label what the service life of a product will be.

5.7.1.2 Products intended for marine use

5.7.1.2.1 Introduction

Raft tests represent worst case conditions with respect to fouling intensity due to their static nature and because the tests are carried out in near shore environments. As the release of active substances from antifouling paints is assisted by hydrodynamic forces (i.e. through polishing), fouling will be more severe on static surfaces compared with moving boats and ships.

Coastal waters are known to have the highest fouling intensity. The littoral zone along coasts constitutes a tiny part of the world's oceans, but contributes markedly to the total marine production. The reason is that benthic production (per unit surface area) exceeds pelagic production by a factor of ten. Coastal macrophytes account for two-thirds of the total biomass of marine photo-synthetic organisms although they can only inhabit less than 0.5 % of the surface area of the oceans[56]. Therefore, when efficacy is demonstrated in coastal waters (the worst case situation), a product is also assumed to be effective in open sea and brackish conditions, and the data can be used to support these uses.

[56] R.S.K. Barnes and R.N. Hughes. An introduction to Marine Ecology. Blackwell Scientific Publications, 1986. Page 37-39

5.7.1.2.2 Dossier requirements

A report of the results from efficacy testing may also include the following about the test site, the test procedures, and the data reported:

- Method of application and information on the panel type and panel preparation;
- Location, geography, and water exchange conditions;
- Water temperature and salinity, including seasonal variations;
- Orientation, dimensions, and exposure depth of the test surface;
- Dimensions and type of material of test panels;
- Identity of the tested product and the control(s);
- Details on the panel preparation (application technique, possible primer paint, paint film thickness, number of coats);
- Date and duration of test;
- Date and raw data from each individual assessment of a test panel;
- Photos of test panel and control(s);
- The overall fouling assessment rating at each inspection during the exposure period;
- A description of the reporting company's weighting system used to provide the overall fouling assessment rating. This should include how fouling coverage has been weighted in order to provide an overall efficacy assessment. The description should be transparent and explicitly explain the calculations carried out. (See example in Appendix 22);
- An interpretation of the data including a conclusion and a discussion of the validity of the results relative to the unprotected reference and the label claim for the product tested.

5.7.1.2.2.1 Testing and field trials

The recommended method for demonstrating efficacy of marine antifouling products is static raft testing. Raft testing allows a high number of formulations to be tested at worst case conditions.

At least one raft test in European coastal waters should be provided. Test in Atlantic or Northern European Seas are preferred; however, other European waters are acceptable too. It is preferable to also provide the reports from additional tests, although these additional tests can be performed in other locations (e.g. in Europe or elsewhere in the world). At least three replicate panels should be provided per product (see section 5.7.1.1.8.4 for more information on replication of tests). Tests should be performed for at least one fouling season, which is at least six months covering the period of peak fouling activity.

5.7.1.2.3 Assessment of authorisation

The ability a product has to produce an antifouling effect is determined by a combination of the activity of the active substance(s) and the mechanical/physico-chemical properties of the paint. Parameters that will define the efficacy of an antifouling product include:

- The potency and release rate of the active substance(s)
- Operational patterns (e.g. speed, idle times, dry-docking interval, etc.)
- Physico-chemical conditions of the water and other climatic, seasonal, or local factors affecting fouling intensity (e.g. concentration of nutrients, hours of daylight, salinity, temperature, presence of ice, turbidity, etc.)

The efficacy data submitted in support of an application represent part of the information assessed to establish if the product has the claimed level of efficacy. It is recognised that the actual in-service performance of an antifouling product will be dependent on a range of factors, which may include how and where a boat or vessel is operated, seasonal and annual variations, as well as the specifics of the antifouling coating itself. Commercial vessels receive tailor-made product specifications in order to meet various planned (and unforeseen) operational conditions. Thus, the general efficacy of a product under typical fouling conditions according to criteria in paragraph 2.3.1 should be demonstrated.

5.7.1.2.3.1 Norms and criteria

The purpose of an efficacy test is to support the label claim. Efficacy is evaluated by comparing the extent of fouling on the test substrate with the fouling on a similar, but unprotected substrate which has been exposed simultaneously and at the same site.

Fouling coverage is frequently evaluated based on the coverage of the typical marine fouling species such as slimes, algae and animals (barnacles, mussels, etc.).

The three types of fouling species (slime, macro-algae and animals) may be rated differently when merged to an overall fouling assessment for the tested product since slime fouling is less significant compared to macro-fouling (for instance for the fuel consumption and manoeuvrability of a ship). An overall fouling assessment may describe the efficacy of a panel in categories such as for instance: 'Excellent', 'Good', 'Fair', and 'Poor'. An example to illustrate how the coverage of the main categories of fouling may be combined to provide an overall fouling assessment is given in Appendix 22.

Since different companies may use different overall fouling assessment systems and interpretation of the result may vary with the type of product (what is 'poor' efficacy for marine water vessels might be 'good' for fresh water yachts), these ratings are not used as the pass/fail criterion for authorisation. Instead, the percentage fouling on the control and test panels is used.

Normally, when tested in marine waters, the negative control will have at least 75 % fouling coverage at the end of the test. In this case, the result from a product under test should be acceptable if the coverage of macro-fouling on the panels is below 25 %. Macro-fouling is defined as large, distinct multicellular organisms visible to the human eye such as barnacles, tubeworms, or fronds of algae[57]. Algae shorter than 5 mm should be regarded as micro-fouling, together with slimes.

If the 25 % criterion is not met, a justification should be provided for why the product may still be regarded as sufficiently efficacious for the intended use.

5.7.1.3 Products for freshwater use

5.7.1.3.1 Introduction

Fresh and brackish waters are known to represent a less severe fouling challenge compared to marine waters. Effective antifouling protection may be environmentally important even where the general fouling challenge is low. For example, to reduce the risk of translocating invasive species (such as zebra mussels) into or between inland waterways, lakes, or brackish seas.

5.7.1.3.2 Dossier requirements

See 5.7.1.2.2 for the requirements on reporting the test procedure and data.

5.7.1.3.2.1 Testing and field trials

For products intended for use in both fresh water and marine waters, a raft test in marine coastal water is sufficient and a separate efficacy test under fresh water conditions is not normally carried out for. Since fresh and brackish waters are known to represent a less severe fouling challenge compared to marine waters, it is common practice to use the bridging principle and refer to tests conducted in marine waters.

For products only intended to be used in fresh water, at least one raft test in fresh water should be provided. When raft tests are carried out in fresh water, the test site should be one known to have relatively high fouling levels, preferably in an area where zebra mussels are present. However, it is preferable to also provide the reports from additional tests. At least three replicate panels should be provided per product (see section 5.7.1.1.8.4 for more information on replication of tests). Tests should be performed for at least one fouling season, which is at least six months covering the period of peak fouling activity.

5.7.1.3.3 Assessment of authorisation

See section 5.7.1.2.3.

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[57] IMO's 2011 Guidelines for the Control and Management of Ship's Biofouling to Minimize the Transfer of Invasive Aquatic Species, Section 2.1. Definitions.

5.7.1.3.3.1 Norms and criteria

The purpose of an efficacy test is to support the label claim. Efficacy is evaluated by comparing the extent of fouling on the test substrate with the fouling on a similar, but unprotected substrate which has been exposed simultaneously and at the same site.

In the case that an efficacy test is carried out in fresh water, it should be noted that as the fouling challenge is low, a 75 % or more coverage of fouling organisms on a negative control test panel cannot be expected. Therefore, if a test is carried out where micro-fouling is predominant and the coverage of macro-fouling is less than 75 %, the test may still be valid. In the case where less than 75 % of the surface of the negative control is covered with fouling, an explanation should be provided for why the test should be considered valid.

It is also possible that in freshwater, macro-fouling (such as freshwater hydrozoans or zebra mussels) may completely cover a negative control.

For tests in fresh water where the control panel has 75 % or more coverage of fouling organisms, the result from a product under test should be considered acceptable if the coverage of macro-fouling on the panels is below 25 %.

For tests in marine water see Section 5.7.1.2.3 for criteria.

5.7.1.4 Products for use in aquaculture

5.7.1.4.1 Introduction

In aquaculture use, antifouling products are used to treat infrastructure, including immersed structures such as cages, nets, ropes, buoys and pontoons, as well as equipment such as pipelines, pumps, filters, and holding tanks.

5.7.1.4.2 Dossier requirements

See 5.7.1.2.2 for the requirements on reporting the test procedure and data.

5.7.1.4.2.1 Testing and field trials

Relevant field or simulated-use trials should be provided to demonstrate the efficacy under in-use conditions. Static testing closely resembles real life conditions for aquaculture use. Test surfaces may include panels and net/cage samples suspended securely from the raft.

At least one field trial should be provided. However, it is preferable to also provide the reports from additional tests. At least three replicates should be provided per product (see section 5.7.1.1.8.4 for more information of replication of tests). Tests should be performed for at least one fouling season, which is at least six months covering the period of peak fouling activity.

5.7.1.4.3 Assessment of authorisation

The ability a product has to produce an antifouling effect is governed by mechanical and physico-chemical properties of the paint. Relevant parameters to be taken into account when assessing the efficacy of an antifouling product include:

- The potency and release rate of the active substance(s) in the paint
- Physico-chemical conditions of the water and other climatic, seasonal or local factors affecting fouling intensity (e.g. concentration of nutrients, hours of daylight, salinity, temperature, presence of ice, turbidity, etc.)

A report of results from efficacy testing should include the following information about the test site, the test procedures, and the data reported:

- Method of application (e.g. dipping of nets) and type of test substrate
- Location, geography, and water exchange conditions
- Water temperature and salinity
- Orientation, dimensions, exposure depth of test surface, and date and duration of the test
- The extent and main categories of fouling and an interpretation of this relative to an unprotected surface and the label claim for the product tested

5.7.1.4.3.1 Norms and criteria

The purpose of an efficacy test is to defend the label claim. Efficacy is evaluated by comparing the extent of fouling on the test substrate (panel, cage, net, etc.) with the fouling on a similar,

but unprotected substrate which has been exposed simultaneously and at the same site. Efficacy is demonstrated if fouling on the treated surface is considerably reduced compared to the fouling on the unprotected surface.

Fouling coverage is frequently evaluated based on the coverage of typical fouling species. These ratings are then merged to provide a consolidated figure for the three major types of fouling species: slime, macro-algae and animals (see Appendix 22). The three types may be rated differently when combined to an overall fouling assessment for the tested product. For example, slime fouling is less significant compared to macro-algae and large hard animals for the water exchange through nets and cages.

If a product for aquaculture use is tested on panels, the pass/fail criteria for the test may be the same as in paragraph 5.7.1.2.3.

5.7.2 PT22 Embalming and taxidermist fluids

5.7.2.1 General introduction

Annex V of BPR defines Product Type 22 products as follows: "Embalming and taxidermist fluids. Products used for the disinfection and preservation of human or animal corpses, or parts thereof". Embalming for this purpose only aims at the temporary preservation of the deceased person, before burial. Taxidermy fluids and those intended for long-term preservation (e.g. repatriation as shipping cases) are not covered by this guidance document. These particular cases will be taken into account in a future update and inclusion into Volume II Part B of the new BPR guidance structure.

This guidance document is intended for applicants to assist them in compiling an authorisation request dossier regarding the efficacy aspect, and thus specifies the general conditions for carrying out efficacy assessments of biocidal products for marketing authorisations.

This guidance document may be reviewed in the event of regulatory changes or technical advances.

5.7.2.2 Use of the products

5.7.2.2.1 The issue of bodily decomposition

5.7.2.2.1.1 Physical, chemical and microbiological post-mortem activities

A body starts to decompose as soon as the blood ceases to circulate and oxygen is no longer supplied to the tissues. Under conditions favourable to decay, the body cools in the first few hours after death, dehydration sets in (lividity) together with rigor mortis resulting from anaerobic hydrolysis of muscle glycogen. The first stages of cell degradation can be seen with the onset of lividity.

The natural degradation of the body's organic matter results from the action of enzyme, tissue and microbial processes. The ecosystem whose characteristics determine the succession of physical, chemical and microbiological changes that occur post-mortem can be defined as the set of interactions between ambient factors (temperature, hygrometry), individual factors, especially the body's water, muscle and fat composition, and the body's own microbial flora, both external (skin) and internal (digestive and respiratory). Together, these conditions affect the establishment, acclimatisation and development of the dominant indigenous flora, separately or in association, and thus steer the metabolism towards either speed or slow decomposition.

The activity of the microflora, initially latent, intensifies; the first stages of mineralisation of the organic matter, stages of the nitrogen, carbon, oxygen and hydrogen cycles, constitute both superficial and profound decomposition. This decay is defined partly by the decomposition of the organic tissues, mainly under the influence of the bacteria hosted by the individual, especially those in the intestinal flora, and then by fungi, and partly by the decomposition of the organic matter and the bacteria responsible for mineralisation that gradually invade the body, via the body fluids.

As the proteins, lipids and certain carbohydrates that provide the substrate degrade; they produce malodorous soluble and gaseous substances, containing sulphur, nitrogen and carboxylates. Depending on the specific activities developed by the flora in place, the resulting

foul odours can vary in nature and intensity. It is increased by higher temperatures and by interferences between chemical groups. As degradation progresses, the source of foul odours moves gradually from the body itself to the liquid products of decay, which rapidly become the principal source of foul odours. As the organic matter becomes hydrolysed into more soluble compounds it becomes easier for microorganisms to assimilate them, facilitating the production of foul odours.

5.7.2.2.1.2 The micro-organisms involved

- in the early stages of decomposition of the liquids and soft tissues (with production of gases), only the following species are found: *Pseudomonas fluorescens* and *Micrococcus ureae*;
- at a later stage of lipid transformation, the following appear: *Pseudomonas* sp. and then *Pyogenes* sp.

The initial wave consists of aerobic bacteria while those following are anaerobic (*Diplococcus magnus, Streptococcus* sp., *Serratia liquefaciens, Bacteriodes* sp. *etc.*). This decomposition of the body due to bacteria and saprotrophic fundi gradually leads to autolysis of the remains, which is pursued later and over time by the bacteria active in the mineralisation of the organic matter, although this last stage is related to the level of humidity. Various factors concerning the environment of the body intervene (humidity, temperature, aeration) as well as its size, age, causes of death and place of storage.

The decay is predominantly influenced by the bacteria that had been hosted by the individual, especially those in the intestinal flora. The bacterial species frequently found in decomposing bodies are:

- of intestinal origin: enterobacteria, especially *Escherichia coli*; clostridia, especially *Clostridium tetani, C. welchii* and *C. difficile*; and faecal *Streptococcus*;
- of dermal origin: *Staphylococcus* spp.;
- of environmental origin: *Bacillus* spp.

The saprotrophic fungi and yeasts succeed one another in specific groups and the flora changes in line with the gradual alteration of the substrate, which thus provides a choice habitat for certain species of mycota at one moment and not at others.

The decomposition of the body due to bacteria and saprotrophic mycota accelerates the alteration started by autolysis, before the mineralising bacteria that invade the body later bring it into the cycle of waste material in the biosphere.

There may also be other pathogenic micro-organisms, such as the tuberculosis bacillus (*Mycobacterium tuberculosis*) or other mycobacteria, or again viruses such as hepatitis or Human Immunodeficiency Virus (HIV), which can persist in the body.

5.7.2.2.2 Products for preserving human bodies and their uses

5.7.2.2.2.1 Types of application

The embalmer begins by physically working the limbs to reduce lividity and facilitate the flow of the preserving fluid. This is used for two separate purposes and at different concentrations:

- arterial fluid: an aquaeous solution injected under pressure into the vascular system (the embalmer adjusts the final concentration to the condition of the body). This liquid is injected in the arterial system via the carotid or the femoral artery (sometimes at several points if diffusion is poor). The injection is made under pressure (by pump) or by gravity. This result in venous drainage: replaced by the injected product, the blood leaves the body via the jugular vein. Six to ten litres are injected and four litres (of blood and other body fluids) are removed by suction;
- cavity fluids: these are usually used at high concentration to preserve the thoracic and abdominal cavities, which cannot be irrigated by arterial injection. Using a trocar connected to a pump, about two litres of the pure undiluted solution are injected into the peritoneal cavity through an incision close to the navel.

There are also preparations for dermal use. These are gels designed to limit the decomposition of the body by treating bedsores. For this type of product, applicants must complete the appropriate section of the assessment grid, demonstrating the efficacy of the product.

In addition to its biocidal active substance(s), such a formulation could include the following co-formulants, which must have no biocidal activity:

- anticoagulants: to fluidify the product and ensure correct diffusion (sodium chloride and sodium citrate);
- hydrating and moistening agents: to slow the drying out of the body by hydrating the tissues and making them more supple (glycerine, ethylene glycol, propylene glycol, hexylene glycol, urea);
- surface-active agents: to facilitate adsorption of the fluid and penetration of the membranes and to maintain the solubility of the other components of the formulation, which are generally cations, as these surfactants are often also antimicrobials;
- colouring agents: to ensure that the fluid is of a colour similar to blood; synthetic colouring agents are generally used (eosin, erythrosine or food colouring agents);
- perfumes.

5.7.2.2.2.2 Products used for aesthetic purposes

Preservation may be supplemented with aesthetic treatment involving remodelling the face (modelling wax), sewing or bonding together the upper and lower jaws, placing eye caps under the eyelids to keep the eyes closed (or possibly gluing them shut). Finally, when all other treatment has been completed, cosmetic make-up may be applied, partly to give a more agreeable appearance but also partly to delay dehydration.

These products are not considered during assessment of the efficacy of the preservation product. However, if these products contain substantial amount of active substance and claim an effect on bodily composition, they should be considered as biocide.

5.7.2.3 Data required

5.7.2.3.1 Claims and labelling

When an application for the approval of a PT 22 substance is being assessed, the evaluation of the efficacy is focused on the efficacy of the biocidal product and not on the other products (as cosmetic) which can be also included in an embalming treatment, so this aspect must be demonstrated unambiguously in laboratory tests and tests on human bodies, the details of which must be available on request.

As a minimum, a PT 22 product must claim to be active against a broad spectrum of bacteria; yeasts, fungi and viruses are considered as an additional spectrum. As explained above, bacteria are the principal micro-organisms targeted by PT 22 products. Yeasts, fungi and viruses have less relevance in the early stages of bodily decomposition.

Nonetheless, an active substance with a broad spectrum on different types of micro-organism would provide better protection for users (e.g. against tuberculosis bacilli, hepatitis viruses or HIV, etc.).

5.7.2.3.2 Efficacy tests

5.7.2.3.2.1 Laboratory tests

As there is currently no standardised method recognised at European level targeting the scope covered by PT 22 products, and as no technical reference documents were found either in France or throughout the world, it is important that methods used should achieve two different yet complementary goals:

- the rapid destruction of bacteria, representative of the bacterial sphere, in the presence of a strongly interfering organic load simulating the bodily fluids;
- to maintain this antibacterial activity for several days, thus demonstrating that there is no subsequent proliferation of these micro-organisms.

5.7.2.3.2.2 Determining bactericidal activity

As already mentioned above, the minimum claim is a bactericidal activity. Other additional activities, such as fungicide or virucide activities must be supported by relevant tests.

From among the techniques available, the selection was made based on the following criteria:

- a method that has been standardised at least at European level – the bacterial "suspension" test used in the medical sector
- the presence of a standardised strong organic load accurately simulating organic bodily fluids.

In compliance with the classification of European standards (EN 14885), the two tests selected belong to the categories of tests in Phase 2, Step 1 which include quantitative suspension tests for establishing that a biocidal product has a bactericidal activity by simulating its use under real conditions:

a) tests according to the EN 13727 standard: this mandatory test determines the minimum bactericidal concentration of a product on the basis of a 5 lg reduction in titre of a bacterial suspension, at a temperature of 20°C, for 60 minutes of contact, in the presence of a strong organic load (bovine albumin 3 g/L + ovine erythrocytes 3 ml/L), on three species of bacteria (*Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541);

b) tests according to the EN 14348 standard: this additional test must be taken into account if the applicant advances any claim concerning activity against agents responsible for tuberculosis, or if complementary tests prove necessary to cover this particular need. This test has a methodology similar to that for the previous test, determining the minimum tuberculocidal concentration of a product on the basis of a 4 lg reduction in titre of a bacterial suspension, at a temperature of 20°C, for 60 minutes of contact, in the presence of a strong organic load (bovine albumin 3 g/L + ovine erythrocytes 3 ml/L), on the bacterium *Mycobacterium terrae* ATCC 15755.

Any claim by applicants that a product targets a specific micro-organism must be supported by supplementary studies. For example, a claim of activity against the agents responsible for tuberculosis must be verified in compliance with the EN 14348 standard. If there is no recognised standard for a specific micro-organism, the EN 14348 standard may be used for the micro-organism in question.

The most recent version of standards in force at the time of the tests must be used.

Furthermore, in accordance with the conclusions in Annex VI (77) *the level, consistency and duration of protection, control or other intended effects must, as a minimum, be similar to those resulting from suitable reference products, where such products exist, or to other means of control. Where no reference products exist, the biocidal product must give a defined level of protection or control in the areas of proposed use*.

Considering the history of the use of formaldehyde, it may therefore be worthwhile to include with the application information about the bactericidal efficacy of formaldehyde, if available.

In France, formaldehyde is most commonly used at concentrations of about 28% for cavity fluid and 1.5% for arterial fluid. As formaldehyde is currently under assessment in the review programme, efficacy data may become available when the assessment report is published by the eCA. The standards proposed above for validating claims may be reviewed at a later stage in the context of the review of this guidance document as a result of the conclusions published by the eCA on the efficacy of formaldehyde, or in the event of other data for this same substance becoming available in the future.

5.7.2.3.2.3 Verifying that antibacterial activity is maintained

When embalming, the biocidal product must remain effective over several days, until burial. The persistence indicated on the label must be proven, e.g. by challenge tests. The following protocol may be used, adapted from the French NF X30-503 standard (Healthcare waste - Reduction by disinfection pre-treatment appliances in microbiological and mechanical risks involving infections and other comparable healthcare waste).

- In order to ensure that bacteria are destroyed and not merely subjected to stress or inhibition by the biocidal product, and to confirm the absence of bacterial revival, the bacterial suspension, treated according to the EN 13727 standard, is held at ambient temperature for four to six days and then the bacteria are counted. In the laboratory, it is held at 20°C until analysis.
- The bacteria in the bacterial suspension are counted on the day of treatment and again after four to six days.

- Lasting disinfection is shown by the absence of bacterial revival, i.e. the bacterial count on day 4-6 must not be increased by more than one lg compared to the bacterial load measured in the sample taken on the day of treatment (Day 0).
- The "effective" dose of the product must be in a range bounded by upper and lower limits, which are:
  - a lower concentration for which bacterial recrudescence is observed after 4-6 days;
  - a higher concentration.

5.7.2.3.2.4 Tests on human bodies

To complement *in vitro* efficacy tests for the biocidal product used for the preservation of human bodies, tests on bodies are necessary to assess product performance.

Because of the number of factors that can influence the efficacy of a biocidal product, such as the cause of death or the time lapsed or the condition of the body before embalming begins, a sufficient number of bodies (at least 20) satisfying the requirements of the grid in Appendix 23 and the claims for the product, must be available for optimum assessment of the results in terms of preservation of the body for viewing by families.

NOTE to the reader:
The applicant has to inquire about the legislation in force in the Member State (MS) where the tests on human bodies are performed (e.g. current French regulations only allow bodies donated to science to be used to test a product that has not yet been approved.

Every centre for the donation of bodies participating in these tests on human bodies must declare the number of bodies undergoing tests in its establishment. This declaration is supplied to the applicant and must be submitted with the application).

In all cases, whatever the legislation in force in each MS, tests on human bodies with good quality and in line with this guidance will be accepted by MS when the dossier will be submitted for authorisation.

The assessment grid for specific biocidal products is shown in Appendix 24. Its purpose is not to assess the overall embalming treatment but only the biocidal product for which authorisation is being requested.

The grid consists of:

- general information: date and place of the treatment, identification of the deceased (gender, age), weight, corpulence, adiposity, date and causes of death, etc.;
- the preoperative body examination: bodily integrity, autopsy, external prostheses, surgery, visible anomalies (decomposition, rigidity, dehydration, lividity, colouring of tissues, dermal lesions, distension of the abdomen, bruising, etc.). The bodies used must be representative of the range of criteria listed in this section;
- the techniques used to inject the biocidal product: timetable, sites and types of injection, biocidal product used, drainage and puncture;
- observations concerning the injection of the biocidal product: observations during treatment, 48 hours after treatment and after different periods in accordance with the applicant's claims;
- where necessary, the use of other products during the preservation process: products for cosmetic purposes, humidifiers and other products.

The embalmer thus assesses the efficacy of the embalming product on a series of human bodies, using the grid provided. The efficacy is judged for the duration claimed by the manufacturer according to observations concerning odour, colouring and the suppleness of the skin after injection of the biocidal product. In the event that the tests on these human bodies have to be interrupted for any reason, the results already obtained remain valid for three years following the official decision to halt the tests.

5.7.2.3.2.5 Choice of dose

The usage dose[58] claimed is a matter for the applicant. Indeed, related to the body conditions, it can be necessary to test several doses above the dose determined in laboratory and then define a range of doses, adapted to difficult cases. They must choose the usage dose claimed according to the efficacy sought and the precautions for use that will be imposed on embalming technicians by their employers, depending on the health risks created by the full preparation (active substance at the chosen concentration plus excipients and solvents). In cases where little is known about the pathogenic micro-organisms that might present a risk to the embalmer, it is essential that protective measures be taken during the preservation process. These measures should not be primary criteria for choosing the biocidal product used for the treatment.

If the applicant chooses a range of doses instead of a single value, the lower must be justified with appropriate tests, as defined in the preceding section (and also the higher dose in the case where different doses have been tested in the human body tests to cover difficult cases). The applicant may also request approval for two different doses, one of them more concentrated for special or difficult cases (bodies found some time after death or in contact with water, for example).

5.7.2.4 Assessing the application for authorisation

The assessment of the embalming product shall be favourable if it satisfies the following efficacy criteria:

- laboratory test: bactericidal properties (EN 13727 and/or EN 14348 standards): obligatory test conditions;
- laboratory test: e.g. challenge test: no bacterial recrudescence for at least 4-6 days by more one lg compared to the bacterial load measured in the sample taken on the day of treatment (Day 0), with the bacterial suspension being held at ambient temperature;
- field trial: 80% of the bodies must meet the satisfaction criteria at T+48 hours. Satisfaction criteria are according to the grid: normal or fair odour, colouring and suppleness of the skin, related to the initial conditions of the body.

SUMMARY OF THE PARAMETERS ASSESSED

EFFICACY CLAIMS ON THE LABEL SUBMITTED

1. Does the applicant make any specific claims? Y/N

2. Have the efficacy claims on the label been judged and dealt with according to the parameters described in this guidance document for this type of product? Y/N

ASSESSING THE DATA

3. Has each study (or supplementary item) been assessed individually for robustness? Y/N

4. Has each study (or supplementary item) been assessed individually for quality assurance? Y/N

5. Has each study (or supplementary item) been assessed individually for suitability (i.e. for reliability and relevance concerning the claims)? Y/N

DECISION-MAKING

Considering all the available data:

6. Are the claims on the label sufficiently supported? Y/N

7. Do the claims on the label require modifications? Y/N

8. On the basis of the efficacy data submitted, can authorisation for the use of the product be recommended? Y/N

[58] Concentration and volume injected

Appendix 1. Claims Matrices

The claims matrices are a set of tables linked to this guidance document: these documents are available on the ECHA Biocides Efficacy Working Group webpage [http://echa.europa.eu/about-us/who-we-are/biocidal-products-committee/working-groups/efficacy.].

The claims matrices linked to this document are intended to cover biocidal products covered under the scope of Product Type 1, 2, 3 and 4 and for Treated Articles.

The claims matrix is a tool for the applicant and CAs. It is intended to capture the information that is needed in the authorisation dossier, to adequately describe typical combinations of products, formats of application of the products, as well as target sites. It also includes the claims made and the requirements for testing these claims (in terms of methodology and appropriate performance standards) for a product to be used in this way.

The reader should note that the matrices are not exhaustive in terms of use patterns, scenarios and test methods.

The claims matrix must be used together with the relevant sections within the efficacy guidance document so as to provide both applicants and CAs alike with clear direction as to the nature and extent of the efficacy data required to support a claimed effect. The claims matrix acts as a guide to the information required when compiling an efficacy dataset for a PT1, PT2, PT3 or PT4 biocidal product and for Treated Articles.

To note:

- Each row (entry) within the matrices is not independent and can be linked to other entries.
- These matrices only address biocidal claims made for these products.
- The claim matrix will be updated regularly according to the state-of-the-art.

Appendix 2. Standards and testing methods for efficacy-testing of disinfectant biocidal products (PT 1-5)

The methods for testing efficacy referenced within this guidance document are enlisted below. The use of European Standards (Table 40) is highly recommended if available and appropriate for the respective application[59]. Should no European Standard for an application be available yet and an adaption of an existing standard is not possible according to the rules laid down in EN 14885, other test methods and guidance documents (Table 41) may be used. In cases where the below mentioned methods are inappropriate to demonstrate efficacy of a product for special applications, methods from other national or international standardisation bodies may also be employed. These include for example, OECD, ASTM or ISO methods. It is recommended to agree such testing strategies with the evaluating CA before tests are performed.

Tests should be carried out according to the respective latest edition of a standard. Please check the respective web sites for the latest information.

Table 40: CEN European standards

Reference	Title	PT	Scope/Remarks
EN 1276	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 1)	1, 2, 4	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 1499	Chemical disinfectants and antiseptics - Hygienic handwash - Test method and requirements (phase 2, step 2)	1	This European Standard specifies a test method simulating practical conditions for establishing whether a hygienic handwash product reduces the transmission of transiently contaminating micro-organisms when used to wash the artificially contaminated hands of volunteers.
EN 1500	Chemical disinfectants and antiseptics - Hygienic handrub - Test method and requirements (phase 2, step 2)	1	This European Standard specifies a test method simulating practical conditions for establishing whether a hygienic handrub product reduces the transmission of transiently contaminating micro-organisms when rubbed onto the artificially contaminated hands of volunteers.
EN 1650	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2, step 1)	1, 2, 4	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

[59] The CEN does not sell or distribute standards or any other deliverable. All European Standards (EN) and drafts (prEN) as well as other approved documents are directly available for purchase from the CEN national standardisation bodies.

Reference	Title	PT	Scope/Remarks
EN 1656	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 1657	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area – Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 12353	Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal (including *Legionella*), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity	1, 2, 3, 4, 5	This method specifies how to keep test organisms used and defined in European Standards for the determination of bactericidal, mycobactericidal, sporicidal, fungicidal and virucidal (incl. bacteriophages) activity of chemical disinfectants and antiseptics drawn up by CEN/TC 216.
EN 12791	Chemical disinfectants and antiseptics - Surgical hand disinfection - Test method and requirements (phase 2, step 2)	1	This European Standard specifies a test method simulating practical conditions for establishing whether a product for surgical hand disinfection reduces the transmission of the microbial flora on hands when used for the treatment of clean hands of volunteers.
EN 13610	Chemical disinfectants - Quantitative suspension test for the evaluation of virucidal activity against bacteriophages of chemical disinfectants used in food and industrial areas - Test method and requirements (phase 2, step 1)	4	This European Standard specifies a method for testing virucidal activity against bacteriophages by assessing reduction in the number of infectious bacteriophage particles in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13623	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity against *Legionella* of chemical disinfectants for aqueous systems - Test method and requirements (phase 2, step 1)	2, 4, 5	This European Standard specifies a method for testing bactericidal activity against *Legionella* by assessing reduction in the number of viable *Legionella* cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13624	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of fungicidal and yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1)	1, 2	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

Reference	Title	PT	Scope/Remarks
EN 13697	Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements without mechanical action (phase 2, step 2)	2, 4	This European Standard specifies a method for testing bactericidal and/or fungicidal or yeasticidal activity by assessing reduction in the number of viable bacterial cells and/or mould spores and/or yeast cells dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13704	Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)	4, (1, 2, 3)	This European Standard specifies a method for testing sporicidal activity by assessing reduction in the number of viable bacterial endospores in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 13727	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1)	1, 2	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14204	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing mycobactericidal activity by assessing reduction in the number of viable mycobacterial cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14348	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)	1, 2	This European Standard specifies a method for testing mycobactericidal activity by assessing reduction in the number of viable mycobacterial cells in suspension under defined conditions. The method is also applicable to demonstrate tuberculocidal activity only. The approach can be applied to formulated products or to biocidal active substances.
EN 14349	Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)	3	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14476	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (phase 2, step 1)	1, 2, (4)	This European Standard specifies a method for testing virucidal activity by assessing reduction in the number of infectious virus particles in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

Reference	Title	PT	Scope/Remarks
EN 14561	Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)	2	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells dried on a frosted glass carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14562	Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of fungicidal or yeasticidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)	2	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells dried on a frosted glass carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14563	Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area - Test method and requirements (phase 2, step 2)	2	This European Standard specifies a method for testing mycobactericidal activity by assessing reduction in the number of viable mycobacterial cells dried on a frosted glass carrier under defined conditions. The method is also applicable to demonstrate tuberculocidal activity only. The approach can be applied to formulated products or to biocidal active substances.
EN 14675	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)	3	This European Standard specifies a method for testing virucidal activity by assessing reduction in the number of infectious virus particles in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 14885	Chemical disinfectants and antiseptics - Application of European Standards for chemical disinfectants and antiseptics	1, 2, 3, 4, 5	This European Standard specifies the European Standards, i.e. test methods, to which products have to conform in order to support the claims for microbicidal activity which are referred to in this document. It also specifies terms and definitions which are used in European Standards. It is applicable to products for which activity is claimed against the following micro-organisms: vegetative bacteria (incl. mycobacteria and *Legionella*), bacterial spores, yeasts, fungal spores and viruses (incl. bacteriophages).
EN 16437	Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)	3	This European Standard specifies a method for testing bactericidal activity by assessing reduction in the number of viable bacterial cells dried on a wood carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

Reference	Title	PT	Scope/Remarks
EN 16438	Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)	3	This European Standard specifies a method for testing fungicidal or yeasticidal activity by assessing reduction in the number of viable mould spores and/or yeast cells dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 16615	Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4-field test) - Test method and requirements (phase 2, step 2)	2, (3, 4)	This European Standard specifies a method for testing bactericidal and/or yeasticidal activity by assessing reduction in the number of viable bacterial and/or yeast cells dried on a PVC carrier under defined conditions. The test applies to products that are used for disinfecting non-porous surfaces by wiping and includes 'ready-to-use wipes' which are impregnated with a microbicidal solution.
EN 16616	Chemical disinfectants and antiseptics - Chemical-thermal textile disinfection - Test method and requirements (phase 2, step 2)	2, (3, 4)	This European Standard specifies a method for testing microbicidal activity of a disinfection process for the treatment of contaminated textile. The procedure is carried out by using a washing machine and microbicidal activity is assessed as the reduction in the number of viable test organisms, such as bacterial, mycobacterial or yeast cells and mould spores, dried on a cotton carrier under defined conditions.
EN 16777	Chemical disinfectants and antiseptics - Quantitative non-porous surface test without mechanical action for the evaluation of virucidal activity of chemical disinfectants used in the medical area - Test method and requirements (phase 2, step 2)	2, (4)	This European Standard specifies a method for testing virucidal activity by assessing reduction in the number of infectious virus particles dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.
EN 17111	Chemical disinfectants and antiseptics. Quantitative carrier test for the evaluation of virucidal activity for instruments used in the medical area. Test method and requirements (phase 2, step 2)	2	This European Standard a method for testing virucidal activity by assessing reduction in the number of infectious virus particles dried under a glass carrier under defined conditions. The approach can be used to formulated products or to biocidal active substances.
EN 17122	Chemical disinfectants and antiseptics. Quantitative nonporous surface test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area. Test method and requirements (phase 2, step 2)	3	This European Standard specifies a test method for testing virucidal activity by assessing reduction in number of infectious virus particles dried on a steel carrier under defined conditions. The approach can be used to formulated products or to biocidal active substances.
EN 17126	Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants in the medical area. Test method and requirements (phase 2, step 1)	2, (3, 4)	This European Standard specifies a method for testing sporicidal activity by assessing reduction in the number of viable bacterial endospores in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

Reference	Title	PT	Scope/Remarks
EN 17272	Chemical disinfectants and antiseptics. Methods of airborne room disinfection by automated process. Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities	2, 3, 4	This European standard specifies a method for testing bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities of airborne surface disinfection by assessing cells dried on a steel carrier under defined conditions.
EN 17387	Chemical disinfectants and antiseptics - Quantitative test for the evaluation of bactericidal and yeasticidal and/or fungicidal activity of chemical disinfectants in the medical area on non-porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)	2, (4)	This European Standard specifies a method for testing bactericidal and/or fungicidal or yeasticidal activity by assessing reduction in the number of viable bacterial cells and/or mould spores and/or yeast cells dried on a steel carrier under defined conditions. The approach can be applied to formulated products or to biocidal active substances.

Table 41: Other test methods and guidance documents

Reference	Title	PT	Remarks
ASTM E2196	Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with Medium Shear and Continuous Flow Using Rotating Disk Reactor	2, 3, 4	This test method is used for growing a reproducible *Pseudomonas aeruginosa* biofilm in a continuously stirred tank reactor (CSTR) under medium shear conditions. In addition, the test method describes how to sample and analyse biofilm for viable cells. Available via: http://www.astm.org/Standard/ or the national standardisation bodies
ASTM E2274	Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants	2, 3	This test method is designed to evaluate sanitizing/disinfectant laundry detergents/additives for use in top-loading automatic clothes washing operations. This test method is designed predominantly to provide testing with representative vegetative bacteria but can also be designed to accommodate the testing of fungi and viruses.
ASTM E2406	Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants for Use in High Efficiency Washing Operations	2, 3	This test method is designed to evaluate sanitizing/disinfectant laundry detergents/additives for use in high efficiency (HE) automatic clothes washing operations that typically utilize very low wash water volumes. This test method is designed to provide testing with representative vegetative bacteria but can also be designed to accommodate the testing of fungi and viruses.
ASTM E2562	Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor	2, 3, 4	This test method specifies the operational parameters required to grow a reproducible *Pseudomonas aeruginosa* biofilm under high shear. The resulting biofilm is representative of generalized situations where biofilm exists under high shear rather than being representative of one particular environment. Available via: http://www.astm.org/Standard/ or the national standardisation bodies

Reference	Title	PT	Remarks
DIN SPEC 10534	Food hygiene - Commercial dishwashing - Hygiene requirements, testing	4	This document is a summary of the standards DIN 10510, DIN 10511, DIN 10512 and DIN 10522. It specifies hygiene requirements relating to the design, construction and operation of commercial warewashers and in particular provides information on their hygienic and proper operation, on cleaning and disinfection of wash ware and on care and maintenance of the machinery. It describes the methods for testing hygienic operation. Available via: http://www.beuth.de/en/ or the national standardisation bodies
DVG Guidelines	Guidelines for the testing of disinfection procedures and chemical disinfectants; Original title: Richtlinien für die Pruefung von Desinfektionsverfahren und chemischen Desinfektionsmitteln	3, 4	DVG Guidelines specify methods for testing activty of chemical disinfectants against bacteria, yeasts and fungal spores, viruses, and parasites. They apply to the veterinary and the food sector, such as animal husbandry, veterinary practices, meat production/food of animal origin, and large-scale/canteen kitchens (except ward kitchens catering patients). DVG Guidelines are published by the German Veterinary Medical Society (DVG). Available in German via: http://www.desinfektion-dvg.de For livestock area the guidelines are available in English via: https://www.desinfektion-dvg.de/index.php?id=2219
ISO 15883-5	Washer-disinfectors – Part 5: Performance requirements and test method criteria for demonstrating cleaning efficacy	2, 3, 4	ISO 15883 relates to a series of standards that specify the required performance levels of Washer-Disinfectors. Part 5, the Technical Specification (TS), describes a method to generate biofilm formed by *Pseudomonas aeruginosa*. Available via: http://www.iso.org/iso/home.htm or the national standardisation bodies.
ISO 20743	Textiles — Determination of antibacterial activity of textile products		Specifies quantitative test methods to determine the antibacterial activity of all antibacterial textile products including nonwovens
ISO 22196	Measurement of antibacterial activity on plastics and other non-porous surfaces		Specifies a method of evaluating the antibacterial activity of antibacterial-treated plastics, and other non-porous, surfaces of products (including intermediate products).
Nordic Working Paper	Efficacy Assessment of Treated Articles: A guidance	1,2,3,4	The document provides guidance on efficacy testing of biocides used in treated articles. The presence and relevance of existing standard test methods is described and, where they do not exist or where they do not provide sufficient support, the nature of the data required will be described. The document was published by the Nordic Council of Ministers. Open access via: http://www.norden.org/en/publications/publikationer/2014-904/

Reference	Title	PT	Remarks
OECD Series on Biocides No. 1	Guidance Document on the Evaluation of the Efficacy of Antimicrobial Treated Articles with Claims for External Effects	2, 3, 4	The document guidance on efficacy testing of articles treated with antimicrobials and articles modified to exert an antimicrobial effect. http://www.oecd.org/env/ehs/pesticides-biocides/41692131.pdf
OECD Series on Biocides No. 4	Guidance Document for Demonstrating Efficacy of Pool and Spa Disinfectants and Field Testing (Series on Testing and Assessment No. 170 and Series on Biocides No. 4)	2	The document provides guidance on setting up a strategy for efficacy testing of pool and spa disinfectants in a laboratory scale testing phase and a field testing phase in a full-size swimming or spa pool. Open access via: http://www.oecd.org/env/ehs/pesticides-biocides/biocidestestguidelinesandguidencedocuments.htm
OECD Series on Biocides No. 6	Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces (Series on Testing and Assessment No. 187 and Series on Biocides No. 6).	2, (4)	This document describes four quantitative methods for testing bactericidal, mycobactericidal, fungicidal and virucidal activity on steel carriers with high application volumes of liquid products. Open access via: http://www.oecd.org/env/ehs/pesticides-biocides/biocidestestguidelinesandguidencedocuments.htm
OECD Series on Biocides No. 8	Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials (Series on Testing and Assessment No. 202 and Series on Biocides No. 8)	1, 2, 3, 4	The document provides guidance for testing the basic antibacterial performance of porous (textile) and non-porous (plastic) materials that have been treated with a biocide with the intention of introducing antibacterial/hygienic properties into that material. Open access via: http://www.oecd.org/env/ehs/pesticides-biocides/biocidestestguidelinesandguidencedocuments.htm
UBA	Quantitative determination of the efficacy of drinking water disinfectants	5	A detailed simulated-use test method for testing the bactericidal and virucidal activity in drinking water, available at: https://www.umweltbundesamt.de/sites/default/files/medien/374/dokumente/150629_version_2_-_quantitative_determination_of_the_efficacy_of_drinking_water_disinfectants.pdf
VAH Standard methods	VAH certification of chemical disinfection procedures; Original title: VAH-Zertifizierung chemischer Desinfektionsverfahren	1, 2	VAH Standard methods specify methods for testing the activity of chemical disinfectants against bacteria (incl. mycobacteria), yeasts, and fungal spores. They apply to testing products used for disinfection in public facilities (medical and other) and, in the event of substantiated medical indications, also in the private home. VAH Standard methods are published by the Association for Applied Hygiene (VAH). Available in German via: http://www.mhp-verlag.de/en/home/

Appendix 3. Table of Reference Test Organisms (PT 1-5)

This table (Table 42) is given as a general overview of relevant test organisms for testing disinfectants in accordance with the BPR.

This table comprises mainly those reference test organisms that are included in the EN norms covered by EN 14885. Furthermore, strains are listed that are recommended for some uses (e.g. endoparasites from DVG standard).

The reader can check the website of the CEN (European Standardization Organizations): www.cen.eu for new and updated standards.

Since the EN systematics of WG's 1 to 3 does not fit exactly to the BPR PT scheme, in borderline cases an indicated reference test organism might be used for other PTs as well. In cases where there are discrepancies between this ECHA guidance and the guidance in EN 14885, the ECHA Guidance should be followed as the leading guidance.

Tests with test organisms in addition to those mentioned below are acceptable, if adequate scientific evidence is submitted on which the relevance of the test organism to the field of use can be judged.

Key for Table 42:

X = mandatory test organisms to claim activity against the specified micro-organism group;

(X) = mandatory test organism to claim activity against the specified micro-organism group for the specific use described in brackets;

O = additional optional test organism;

* The test organisms are usually the same for phase 2, step 1 suspension tests and phase 2, step 2 tests, but there are exceptions to this rule, e.g.:

- PT5 bactericidal activity: the other bacteria are tested in suspension tests, and *E. coli* A3 and *E. faecium* Teltow 11 in the simulated-use test;
- PT1 and PT2 virucidal activity: Poliovirus is tested only in suspension tests;
- PT3 hard non-porous surfaces and room disinfection virucidal activity: Bovine Enterovirus type 1 (ECBO) in phase 2, step 1 test and Porcine Parvovirus NADL2 in phase 2, step 2 test;
- PT3 hard porous surfaces, animal feet disinfection and disinfection of hatching-eggs, virucidal activity: Bovine Enterovirus type 1 (ECBO) in phase 2, step 1 test, and ECBO, Reovirus type 1, Vaccinia virus strain Elstree ATCC VR-1549 and Newcastle disease virus (ND) strain Montana in phase 2, step 2 test according to the DVG guideline;
- PT4 virucidal activity: in phase 2, step 1 tests Norovirus and Adenovirus should be tested, and in phase 2, step 2 only Norovirus;
- PT5 virucidal activity: in EN suspension tests efficacy against enteroviruses and Norovirus should be tested, and in the simulated-use test against bacteriophages.

Table 42: Reference Test Organisms

Micro-organisms	PT1	PT2	PT3	PT4	PT5
Bacteria					
Staphylococcus aureus ATCC 6538	X	X	X	X	X
Pseudomonas aeruginosa ATCC 15442 (not for teat disinfection)	X	X	X	X	X
Enterococcus hirae ATCC 10541 (not for teat disinfection)	X	X	X	X	X
Escherichia coli ATCC 10536 (PT2: domestic area and industry; PT3 teat disinfection)		(X)	(X)	X	X
Escherichia coli K12 NCTC 10538 (PT2 textiles, EN 16616 test)	X	(X)			
Escherichia coli A3 DSM 110652					X*
Salmonella typhimurium ATCC 13311		O		O	
Lactobacillus brevis DSM 6235		O		O	

Micro-organisms	PT1	PT2	PT3	PT4	PT5
Enterobacter cloacae DSM 6234		O		O	
Enterococcus faecium ATCC 6057 (for T >40°C)		(X)		(X)	
*Proteus hauseri (*ex *P. vulgaris)* ATCC 13315 (not for teat disinfection)			X		
Enterococcus faecium Teltow 11 DSM 110643					X*
Streptococcus uberis ATCC 19436 (teat disinfection)			(X)	O	
Legionella pneumophila ATCC 33152 (PT2: pools, hot tubs; PT4: drinking water systems, PT5: collective drinking water systems)		(X)		(X)	(X)
Legionella pneumophila ATCC 43108		O			O
Acinetobacter baumannii ATCC 19606 (PT 2 room disinfection, medical area)		(X)			
Yeasts					
Candida albicans ATCC 10231	X	X	X	X	O
Saccharomyces cerevisiae ATCC 9763 or *Saccharomyces cerevisiae* DSM 70487 (breweries)				(X)	
Fungal spores					
Aspergillus brasiliensis (ex *A. niger)* ATCC 16404	X	X	X	X	O
Virucidal claim					
Poliovirus type 1, LSc 2ab (Picornavirus)	X*	X*			
Adenovirus, type 5, strain Adenoid 75, ATCC VR-5	X	X		X*	
Murine Norovirus, strain S99 Berlin	X	X		X*	X*
Murine Parvovirus, strain Crawford, ATCC VR-1346 (for T ≥40°C)		(X)		(X)	
Bovine Enterovirus type 1, ECBO ATCC VR-248			X*		
Porcine Parvovirus strain NADL2			X*		
Rotavirus (pools, hot tubs)		(X)			
Enterovirus, e.g. Coxsackievirus B4 or B5					X*
Reovirus type 1 (porous surfaces)			(X)*		
Vaccinia virus strain Elstree ATCC VR-1549 (porous surfaces)			(X)*		
Newcastle disease virus (ND) strain Montana (porous surfaces)			(X)*		
Limited spectrum virucidal claim					
Adenovirus, type 5, strain Adenoid 75, ATCC VR-5	X	X			
Murine Norovirus, strain S99 Berlin	X	X			
Claim against enveloped viruses					
Modified Vaccinia virus Ankara (MVA) ATCC VR-1508, or Vaccinia virus strain Elstree ATCC VR-1549 (teat disinfection)	X	X	(X)		
Bacteriophages					
Bacteriophage P001 DMS 4262 (milk industry)				X	
Bacteriophage P008 DMS 10567 (milk industry)				X	
Bacteriophage MS2 DSM 13767 or ATCC 15597-B1					X*
Bacteriophage PRD1 DSM 19107					X*
Mycobacteria					
Mycobacterium terrae ATCC 15755	X	X			
Mycobacterium avium ATCC 15769	X	X	X		

Micro-organisms	PT1	PT2	PT3	PT4	PT5
(PT1 and PT2 claim for mycobactericidal: both, tuberculocidal: *M. terrae* only)					
Bacterial spores					
Spores of *Bacillus cereus* ATCC 12826 (PT2 depending on use area / PT3 beehives)		O (X)	O (X)	O	
Spores of *Bacillus subtilis* ATCC 6633 (beehives)		X	X	X	
Spores of *Clostridioides difficile* R027 NCTC 13366		O			
Spores of *Clostridium sporogenes* CIP 7939		O	O	O	
Spores of *Geobacillus stearothermophilus* (for T ≥60°C)		O		O	
Endoparasites					
Cryptosporidium parvum strain Leipzig, or other strain with analogous excystation rate and cultivability behaviour			X		
Ascaris suum			X		

Appendix 4. Overview of standards, test conditions and pass criteria (PT 1-5)

The overview is presented in a number of tables. This information should be read together with Appendix 1: Claims matrices and Appendix 3: Table of reference test organisms (PT 1-5).

Please note that this is a simplified overview of the requirements for disinfectant biocides. Always check the respective sections of the guidance and Technical Agreements for Biocides (TAB) for additional requirements. Individual chapters of the guidance provide information on further uses that are not yet reflected in Appendix 4.

It should be noted that although this guidance is mainly based on EN standards, there are some cases where there are discrepancies between the guidance and the EN tests and in such cases the ECHA guidance should be followed as the leading guidance.

The reader is strongly advised to check whether there are new versions of the standards on the website of the CEN: www.cen.eu.

It should be noted that if tests other than CEN standards (notably when no CEN tests are available) are used, and pass criteria are available, these should be met (unless stated differently in this guidance). When the test does not provide pass criteria, the criteria in this table can be taken into account as guidance for what level of reduction is normally required.

In all cases, deviations from these standards are possible but should be justified in the application.

PT 1						
Product type/micro-organism	**Requirements[1]**	**Test required[2]**	**Contact time[3]**	**Temp (°C)[4]**	**Soiling conditions[5]**	**Required lg reduction**
PT 1 Hand disinfection - hygienic handrub[6]						
bacteria	Basic requirement - 2,1 test	EN 13727 / EN 1276[7]	30 - 60 sec[8]	20	clean/dirty	5
bacteria	Basic requirement - 2,2 test	EN 1500	30 - 60 sec[8]	skin T	none	≥ propan-2-ol[9]
yeasts	Basic requirement - 2,1 test	EN 13624 / EN 1650[7]	30 - 60 sec[8]	20	clean/dirty	4
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	30 - 60 sec[8]	20	clean/dirty	4
viruses[10]	If claimed - 2,1 test	EN 14476	30 - 120 sec[8]	20	clean/dirty	4
PT 1 Hand disinfection - hygienic handwash[6]						
bacteria	Basic requirement - 2,1 test	EN 13727 / EN 1276[7]	30 - 60 sec[8]	20	dirty[11]	3[12]
bacteria	Basic requirement - 2,2 test	EN 1499	30 - 60 sec[8]	skin T	none	> control[13]
yeasts	Basic requirement - 2,1 test	EN 13624 / EN 1650[7]	30 - 60 sec[8]	20	dirty[11]	2[12]
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	30 - 60 sec[8]	20	dirty[11]	2[12]
enveloped viruses	If claimed - 2,1 test	EN 14476	30 - 120 sec[8]	20	dirty[11]	2[12]
PT 1 hand disinfection - surgical handrub[6]						
bacteria	Basic requirement - 2,1 test	EN 13727	1-5 min[14]	20	clean	5
bacteria	Basic requirement - 2,2 test	EN 12791	1-5 min[14]	skin T	none	≥ propan-1-ol[15]
yeasts	Basic requirement - 2,1 test	EN 13624	1-5 min[14]	20	clean	4
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	1-5 min[14]	20	clean	4
PT 1 Hand disinfection - surgical handwash[6]						
bacteria	Basic requirement - 2,1 test	EN 13727	1-5 min[14]	20	clean/dirty	5
bacteria	Basic requirement - 2,2 test	EN 12791	1-5 min[14]	skin T	none	≥ propan-1-ol[15]
yeasts	Basic requirement - 2,1 test	EN 13624	1-5 min[14]	20	clean/dirty	4
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	1-5 min[14]	20	clean / dirty	4

PT 2						
Product type / micro-organism	**Requirements[1]**	**Test required[2]**	**Contact time[3]**	**Temp (°C)[4]**	**Soiling conditions[5]**	**Required lg reduction**
PT 2 Hard surface disinfection and other uses where EN tests are applicable, use in healthcare[16]						
bacteria	Basic requirement - 2,1 test	EN 13727 / EN 1276[7]	5 min/ 60 min	20	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 17387 / EN 13697[7] / EN 16615[19]	5 min/ 60 min	20	clean / dirty	5/4/5
yeasts	Basic requirement - 2,1 test	EN 13624 / EN 1650[7]	5 min/ 60 min	20	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 17387 / EN 13697[7]/ EN 16615[19]	5 min / 60 min	20	clean / dirty	4/3/4
fungal spores	If claimed - 2,1 test	EN 13624 / EN 1650[7]	15 min/ 60 min	20	clean / dirty	4
fungal spores	If claimed - 2,2 test	EN 17387 / EN 13697[7]	15 min/ 60 min	20	clean / dirty	4/3
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	15 min/ 60 min	20	clean / dirty	4
bacterial spores	If claimed – 2,1 test	EN 17126 / EN 13704[7]	15 min/ 60 min	20	clean / dirty	4/3
viruses[10]	If claimed - 2,1 test	EN 14476	15 min/ 60 min	20	clean / dirty	4
viruses[10]	If claimed – 2,2 test	EN 16777	15 min/ 60 min	20	clean / dirty	4
PT 2 Hard surface disinfection and other uses where EN tests are applicable, use other than in healthcare						
bacteria	Basic requirement - 2,1 test	EN 13727 / EN 1276[7]	as claimed	as claimed	clean/dirty	5
bacteria	Basic requirement - 2,2 test	EN 13697 / EN 16615[19]	as claimed	as claimed	clean/dirty	4/5
yeasts	If claimed - 2,1 test	EN 13624 / EN 1650[7]	as claimed	as claimed	clean / dirty	4
yeasts	If claimed - 2,2 test	EN 13697 / EN 16615[19]	as claimed	as claimed	clean / dirty	3/4
fungal spores	If claimed - 2,1 test	EN 13624 / EN 1650[7]	as claimed	as claimed	clean / dirty	4
fungal spores	If claimed - 2,2 test	EN 13697	as claimed	as claimed	clean / dirty	3
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	as claimed	as claimed	clean / dirty	4
bacterial spores	If claimed – 2,1 test	EN 17126 / EN 13704[7]	as claimed	as claimed	clean / dirty	4/3
viruses[10]	If claimed - 2,1 test	EN 14476	as claimed	as claimed	clean / dirty	4
viruses[10]	If claimed – 2,2 test	EN 16777 adapted[20]	as claimed	as claimed	clean / dirty	4

PT 2 Room disinfection/automated airborne disinfection of surfaces (including use in healthcare)						
bacteria	Basic requirement - 2,2 test	EN 17272	as claimed/max 48h	20	clean/dirty	5
yeasts	Basic requirement – 2,2 test	EN 17272	as claimed/max 48 h	20	clean / dirty	4
fungal spores	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
bacterial spores	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
mycobacteria / tuberculosis bacteria	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
viruses	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
PT 2 (Instrument) disinfection by immersion or filling, use in medical area and other areas with similar hygienic requirements						
bacteria	Basic requirement - 2,1 test	EN 13727	as claimed	as claimed	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 14561	as claimed	as claimed	clean / dirty	5
yeasts	Basic requirement - 2,1 test	EN 13624	as claimed	as claimed	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 14562	as claimed	as claimed	clean / dirty	4
fungal spores	If claimed - 2,1 test	EN 13624	as claimed	as claimed	clean / dirty	4
fungal spores	If claimed - 2,2 test	EN 14562	as claimed	as claimed	clean / dirty	4
bacterial spores	If claimed – 2,1 test	EN 17126	as claimed	as claimed	clean / dirty	4
mycobacteria / tuberculosis bacteria	If claimed - 2,1 test	EN 14348	as claimed	as claimed	clean / dirty	4
mycobacteria / tuberculosis bacteria	If claimed - 2,2 test	EN 14563	as claimed	as claimed	clean / dirty	4
viruses	Basic requirement - 2,1 test	EN 14476	as claimed	as claimed	clean / dirty	4
viruses	Basic requirement – 2,2 test	EN 17111	as claimed	as claimed	clean / dirty	4

PT 2 (Instrument) disinfection by immersion or filling, other use areas						
bacteria	Basic requirement - 2,1 test	EN 13727 / EN 1276	as claimed	as claimed	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 14561 adapted / EN 13697	as claimed	as claimed	clean / dirty	4¤
yeasts	If claimed - 2,1 test	EN 13624 / EN 1650	as claimed	as claimed	clean / dirty	4
yeasts	If claimed - 2,2 test	EN 14562 adapted / EN 13697	as claimed	as claimed	clean / dirty	3
fungal spores	If claimed - 2,1 test	EN 13624 / EN 1650	as claimed	as claimed	clean / dirty	4
fungal spores	If claimed - 2,2 test	EN 14562 adapted / EN 13697	as claimed	as claimed	clean / dirty	3
bacterial spores	If claimed – 2,1 test	EN 17126 / EN 13704	as claimed	as claimed	clean / dirty	4
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	as claimed	as claimed	clean / dirty	4
mycobacteria/tuberculosis bacteria	If claimed - 2,2 test	EN 14563	as claimed	as claimed	clean / dirty	4
viruses	If claimed - 2,1 test	EN 14476	as claimed	as claimed	clean / dirty	4
viruses	If claimed – 2,2 test	EN 17111	as claimed	as claimed	clean / dirty	4
PT 2 Textile/Laundry process disinfection						
bacteria	Basic requirement - 2,1 test	EN 13727 / EN 1276[7]	as claimed	as claimed	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 16616 / ASTM E2406 / ASTM E2274[22]	as claimed	as claimed	clean / dirty	7/4/4
yeasts	Basic requirement - 2,1 test	EN 13624 / EN 1650[7]	as claimed	as claimed	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 16616 / ASTM E2406 / ASTM E2274[22]	as claimed	as claimed	clean / dirty	6/3/3
fungal spores	If claimed - 2,1 test	EN 13624 / EN 1650[7]	as claimed	as claimed	clean / dirty	4
fungal spores	If claimed - 2,2 test	EN 16616 / ASTM E2406 / ASTM E2274[22]	as claimed	as claimed	clean / dirty	6/3/3
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14348	as claimed	as claimed	clean / dirty	4
mycobacteria/tuberculosis bacteria	If claimed - 2,2 test	EN 16616 / ASTM E2406 / ASTM E2274[22]	as claimed	as claimed	clean / dirty	7/4/4
viruses	If claimed - 2,1 test	EN 14476	as claimed	as claimed	clean / dirty	4
viruses	If claimed - 2,2 test	ASTM E2406 / ASTM E2274[22]	as claimed	as claimed	clean / dirty	3/3

PT 3						
Product type / micro-organism	**Requirements[1]**	**Test required[2]**	**Contact time[3]**	**Temp (°C)[4]**	**Soiling conditions[5]**	**Required lg reduction**
PT 3 Hard surface disinfection – non-porous surfaces						
bacteria	Basic requirement - 2,1 test	EN 1656	as claimed[23]	10	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 14349/EN 16615[19]	as claimed[23]	10	clean / dirty	4
yeasts	Basic requirement - 2,1 test	EN 1657	as claimed[23]	10	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 16438/EN 16615[19]	as claimed[23]	10	clean / dirty	3
fungal spores	If claimed - 2,1 test	EN 1657	as claimed[23]	10	clean / dirty	4
fungal spores	If claimed - 2,2 test	EN 16438	as claimed[23]	10	clean / dirty	3
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14204	as claimed[23]	10	clean / dirty	4
mycobacteria/tuberculosis bacteria	If claimed – 2,2 test	DVG guideline for tuberculocidal efficacy[24]	as claimed[23]	10	clean / dirty	4
bacterial spores	If claimed – 2,1 test	EN 13704	as claimed[23]	10	clean / dirty	3
viruses	If claimed - 2,1 test	EN 14675	as claimed[23]	10	clean / dirty	4
viruses	If claimed – 2,2 test	EN 17122	as claimed[23]	10	clean / dirty	3
endoparasites	If claimed – 2,1 test	DVG guideline for antiparasitic efficacy[24]				
endoparasites	If claimed – 2,2 test	DVG guideline for antiparasitic efficacy[24]				

PT 3 Hard surface disinfection – porous surfaces						
bacteria	Basic requirement - 2,1 test	EN 1656	as claimed[23]	10	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 16437	as claimed[23]	10	clean / dirty	4
yeasts	Basic requirement - 2,1 test	EN 1657	as claimed[23]	10	clean / dirty	4
yeasts	Basic requirement - 2,2 test	DVG guideline for fungicidal efficacy[24, 25]	as claimed[23]	10	clean / dirty	4
fungal spores	If claimed - 2,1 test	EN 1657	as claimed[23]	10	clean / dirty	4
fungal spores	If claimed - 2,2 test	DVG guideline for fungicidal efficacy[24, 25]	as claimed[23]	10	clean / dirty	3
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14204	as claimed[23]	10	clean / dirty	4
mycobacteria/tuberculosis bacteria	If claimed – 2,2 test	DVG guideline for tuberculocidal efficacy[24, 25]	as claimed[23]	10	clean / dirty	4
bacterial spores	If claimed – 2,1 test	EN 13704	as claimed[23]	10	clean / dirty	3
viruses	If claimed - 2,1 test	EN 14675	as claimed[23]	10	clean / dirty	4
viruses	If claimed – 2,2 test	DVG guideline for virucidal efficacy[24, 25]	as claimed[23]	10	as specified in the DVG guideline[17]	4
endoparasites	If claimed – 2,1 test	DVG guideline for antiparasitic efficacy[24]				
endoparasites	If claimed – 2,2 test	DVG guideline for antiparasitic efficacy[24]				
PT 3 Surface disinfection on the outside of animal transportation vehicles – non-porous surfaces						
bacteria, yeasts, fungal spores, mycobacteria / tuberculosis bacteria, endoparasites	As PT 3 hard non-porous surfaces		5 min[23]	As PT 3 hard non-porous surfaces		
viruses	Basic requirement - 2,1 test	EN 14675	5 min[23]	10	clean / dirty	4
viruses	Basic requirement – 2,2 test	EN 17122	5 min[23]	10	clean / dirty	3

PT 3 Surface disinfection on the outside of animal transportation vehicles – porous surfaces						
bacteria, yeasts, fungal spores, mycobacteria / tuberculosis bacteria, endoparasites	As PT 3 hard porous surfaces		5 min[23]	As PT 3 hard porous surfaces		
viruses	Basic requirement - 2,1 test	EN 14675	5 min[23]	10	clean / dirty	4
viruses	Basic requirement – 2,2 test	DVG guideline for virucidal efficacy[24]	5 min[23]	10	as specified in the DVG guideline[17]	4
PT 3 Teat disinfection						
bacteria pre-milking	Basic requirement - 2,1 test	EN 1656	60 sec[26]	30	clean / dirty	5
bacteria pre-milking	Basic requirement - 2,2 test	should be provided[18]	60 sec[26]	30		
bacteria post-milking	Basic requirement - 2,1 test	EN 1656	5 min[26]	30	clean / dirty	5
bacteria post-milking	Basic requirement - 2,2 test	should be provided[18]	5 min[26]	30		
yeasts pre-milking	Basic requirement - 2,1 test	EN 1657	60 sec[26]	30	clean / dirty	4
yeasts post-milking	Basic requirement - 2,1 test	EN 1657	5 min[26]	30	clean / dirty	4
yeasts pre/post-milking	Optional – 2,2 test	See[25]				
fungal spores pre/post milking	If claimed - 2,1 test	EN 1657	60 sec / 5 min[26]	30	clean / dirty	4
fungal spores pre/post milking	If claimed - 2,2 test	should be provided	60 sec / 5 min[26]	30	clean / dirty	4
mycobacteria/tuberculosis bacteria pre/post milking	If claimed - 2,1 test	EN 14204	60 sec / 5 min[26]	30	clean / dirty	4
viruses pre/post milking	If claimed - 2,1 test	EN 14675	60 sec / 5 min[26]	30	clean / dirty	4
algae	If claimed	to be proposed				

PT 3 Animal feet disinfection						
bacteria	Basic requirement - 2,1 test	EN 1656	5 min	10	dirty[27]	5
bacteria	Basic requirement - 2,2 test	EN 16437	5 min	10	dirty[27]	4
yeasts	If claimed - 2,1 test	EN 1657	5 min	10	dirty[27]	4
yeasts	If claimed - 2,2 test	DVG guideline for fungicidal efficacy[24, 25]	5 min	10	dirty[27]	4
fungal spores	If claimed – 2,1 test	EN 1657	5 min	10	dirty[27]	4
fungal spores	If claimed – 2,2 test	DVG guideline for fungicidal efficacy[24, 25]	5 min	10	dirty[27]	3
mycobacteria/tuberculosis bacteria	If claimed - 2,1 test	EN 14204	5 min	10	dirty[27]	4
mycobacteria/tuberculosis bacteria	If claimed – 2,2 test	DVG guideline for tuberculocidal efficacy[24, 25]	as claimed[23]	10	dirty[27]	4
viruses	If claimed - 2,1 test	EN 14675	5 min	10	dirty[27]	4
viruses[10]	If claimed - 2,2 test	DVG guideline for virucidal efficacy[24]	5 min	10	as specified in the DVG guideline[17]	3
PT 3 Disinfection of hatching-eggs						
bacteria	Basic requirement - 2,1 test	EN 1656	as claimed	30	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 16437	as claimed	30	clean / dirty	4
fungal spores	Basic requirement - 2,1 test	EN 1657	as claimed	30	clean / dirty	4
fungal spores	Basic requirement - 2,2 test	DVG guideline for fungicidal efficacy[24, 25]	as claimed	30	clean / dirty	3
other target organisms	If claimed - 2,1 test	As PT 3 porous surfaces				
other target organisms	If claimed - 2,2 test	As PT 3 porous surfaces				

PT 3 Room disinfection/automated airborne disinfection of surfaces in veterinary area						
bacteria	Basic requirement – 2,2 test	EN 17272	as claimed / max 48 h	10	clean / dirty	5
yeasts	Basic requirement – 2,2 test	EN 17272	as claimed / max 48 h	10	clean / dirty	4
fungal spores	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	10	clean / dirty	4
mycobacteria / tuberculosis bacteria	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	10	clean / dirty	4
bacterial spores	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	10	clean / dirty	3
viruses	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	10	clean / dirty	4
PT 3 Textile disinfection						
bacteria	Basic requirement - 2,1 test	EN 1656	as claimed	as claimed	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 16616 / ASTM E2406[21] / ASTM E2274[22]	as claimed	as claimed	clean / dirty	7/4/4
yeasts	Basic requirement - 2,1 test	EN 1657	as claimed	as claimed	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 16616 / ASTM E2406[21] / ASTM E2274[22]	as claimed	as claimed	clean / dirty	6/3/3
other target organisms	If claimed - 2,1 test	As PT 3 hard non-porous surface disinfection				
other target organisms	If claimed - 2,2 test	As PT 2 textile disinfection				
PT 3 Disinfection of beehives and beekeeping equipment						
bacteria	Basic requirement - 2,1 test	EN 1656	as claimed	10	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 16437	as claimed	10	clean / dirty	4
bacterial spores	Basic requirement - 2,1 test	EN 13704	as claimed	10	clean / dirty	4
bacterial spores	Basic requirement - 2,2 test	EN 16437 adapted	as claimed	10	clean / dirty	3
other target organisms	If claimed - 2,1 test	As PT 3 porous surfaces				
other target organisms	If claimed - 2,2 test	As PT 3 porous surfaces				

PT 4						
Product type/ micro-organism	Requirements[1]	Test required[2]	Contact time[3]	Temp (°C)[4]	Soiling conditions[5]	Required lg reduction
PT 4 Hard surface disinfection						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	as claimed	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 13697 / EN 16615[19]	as claimed	as claimed	clean / dirty	4/5
yeasts	Basic requirement - 2,1 test	EN 1650	as claimed	as claimed	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 13697 / EN 16615[19]	as claimed	as claimed	clean / dirty	3/4
fungal spores	If claimed - 2,1 test	EN 1650	as claimed	as claimed	clean / dirty	4
fungal spores	If claimed - 2,2 test	EN 13697	as claimed	as claimed	clean / dirty	3
mycobacteria	If claimed - 2,1 test	EN 14348	as claimed	as claimed	clean / dirty	4
bacterial spores	If claimed - 2,1 test	EN 13704	as claimed	as claimed	clean / dirty	3
viruses	If claimed - 2,1 test	EN 14476 adapted[28]	as claimed	as claimed	clean / dirty	4
viruses	If claimed – 2,2 test	EN 16777 adapted[20]	as claimed	as claimed	clean / dirty	4
bacteriophages	If claimed - 2,1 test	EN 13610[28]	as claimed	as claimed	clean / dirty	4
PT 4 Room disinfection/automated airborne surface disinfection						
bacteria	Basic requirement – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	5
yeasts	Basic requirement – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
fungal spores	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
mycobacteria / tuberculosis bacteria	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
bacterial spores	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	3
viruses	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4
bacteriophages	If claimed – 2,2 test	EN 17272	as claimed / max 48 h	20	clean / dirty	4

PT 4 Disinfection of inner surfaces without circulation						
see PT04 hard surfaces			as claimed	as claimed		
PT 4 Disinfection of inner surfaces by CIP						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	as claimed	clean / dirty	5
yeasts	Basic requirement - 2,1 test	EN 1650	as claimed	as claimed	clean / dirty	4
other target organisms	If claimed – 2,1 test	see PT 4 hard surfaces				
PT 4 Disinfection of inner surfaces in human drinking water systems						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	20	clean / dirty	5
bacteria	Basic requirement – 2,2 test	EN 13697	as claimed	20	clean / dirty	4
Legionella	If claimed - 2,1 test	EN 13623	as claimed	20	clean / dirty	4
Legionella	If claimed – simulated-use test or field trial	See section 5.4.4.6.2. Note that when efficacy against *Legionella* is claimed, both a phase 2, step 1 test, and either a simulated-use test or a field trial is required.				
other organisms	If claimed - 2,1 test	as PT 4 hard surfaces				
other organisms	If claimed – 2,2 test	as PT 4 hard surfaces				
PT 4 Equipment disinfection by soaking						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	as claimed	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 13697	as claimed	as claimed	clean / dirty	4
yeasts	Basic requirement - 2,1 test	EN 1650	as claimed	as claimed	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 13697	as claimed	as claimed	clean / dirty	3
other organisms when claimed	If claimed - 2,1 test	as PT 4 hard surfaces				
other organisms when claimed	If claimed - 2,2 test	as PT 4 hard surfaces				

PT 4 Disinfection of inner surfaces in veterinary water systems						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	10	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 13697	as claimed	10	clean / dirty	4
other organisms	If claimed - 2,1 test	as PT 4 hard surfaces (temperature 10°C)				
other organisms	If claimed – 2,2 test	as PT 4 hard surfaces (temperature 10°C)				
PT 4 Disinfection in dishwashing machines and crate washers						
bacteria	Basic requirement - 2,1 test	EN 1276	as claimed	as claimed	clean / dirty	5
bacteria	Basic requirement - 2,2 test	EN 13697	as claimed	as claimed	clean / dirty	4
bacteria	Basic requirement - 3 test	DIN SPEC 10534	as claimed	as claimed	clean /dirty	
yeasts	Basic requirement - 2,1 test	EN 1650	as claimed	as claimed	clean / dirty	4
yeasts	Basic requirement - 2,2 test	EN 13697	as claimed	as claimed	clean / dirty	3
yeasts	Basic requirement - 3 test	DIN SPEC 10534	as claimed	as claimed	clean / dirty	
other organisms	If claimed - 2,1 test	as PT 4 hard surfaces				
other organisms	If claimed - 2,2 test	as PT 4 hard surfaces				

PT 5						
Product type/ micro-organism	**Requirements[1]**	**Test required[2]**	**Contact time[3]**	**Temp (°C)[4]**	**Soiling conditions[5]**	**Required lg reduction**
PT 5 Disinfection at the drinking water suppliers and their water distribution systems						
bacteria	Basic requirement – 2,1 test	EN 1276 adapted	30 min	15	clean / dirty	5
bacteria	Basic requirement – simulated-use test	Test protocol[29]	10 min / 25 min	15	clean	2 / 4
viruses	Basic requirement – 2,1 test	EN 14476 adapted	30 min	15	clean / dirty	4
viruses	Basic requirement – simulated-use test	Test protocol[29]	10 min / 25 min	15	clean	2 / 4
other organisms	If claimed – 2,1 test					
PT 5 Disinfection of raw water for individual supply (1-2 premises)						
bacteria	Basic requirement – 2,1 test	EN 1276 adapted	30 min	15	dirty	5
bacteria	Basic requirement – simulated-use test	Test protocol[29]	10 min / 25 min	15	clean	2/4
viruses	Basic requirement – 2,1 test	EN 14476 adapted	30 min	15	dirty	4
viruses	Basic requirement – simulated-use test	Test protocol[29]	10 min / 25 min	15	clean	2/4
other organisms	If claimed – 2,1 test					
PT 5 Disinfection in collective drinking water systems						
bacteria	Basic requirement – 2,1 test	EN 1276 adapted	25 min	15	clean	5
Legionella	Basic requirement – 2,1 test	EN 13623	25 min	15	clean	4
Legionella	Basic requirement – simulated-use test	Test protocol[30]	as claimed	15	clean	4
Legionella	Basic requirement – field trial	See Guidance: Vol II B+C, section 5.4.5.4.2 (Test conditions/Field Trials)				
other organisms	If claimed – 2,1 test					
other organisms	If claimed – simulated-use test or field trial					

PT 5 Disinfection of water in reservoirs						
bacteria	Basic requirement – 2,1 test	EN 1276 adapted	as claimed	15	clean / dirty	5
bacteria	Basic requirement – simulated-use test	Test to be developed	as claimed	15	clean / dirty	Criteria for drinking water according to the DWD should be met
viruses	Basic requirement – 2,1 test	EN 14476 adapted	as claimed	15	clean / dirty	4
viruses	Basic requirement – simulated-use test	Test to be developed	as claimed	15	clean / dirty	Criteria for drinking water according to the DWD should be met
other organisms, e.g. *Legionella*	If claimed – simulated-use test					
PT 5 Disinfection of water of undefined quality for small scale use (up to 5 L/person/day)						
bacteria	Basic requirement – 2,1 test	EN 1276 adapted	30 min	15	dirty	5
viruses	Basic requirement – 2,1 test	EN 14476 adapted	30 min	15	dirty	4
other organisms	If claimed – 2,1 test		30 min, unless a longer CT is justified			
all organisms	Basic requirement - field trial (if no pre-treatment for turbidity)		30 min, unless a longer CT is justified			
PT 5 Disinfection of water for animals						
bacteria	Basic requirement – 2,1 test	EN 1276 adapted	30 min[31]	as claimed	clean / dirty	5
bacteria	Basic requirement – simulated-use test or field trial	Test protocol[29] or field trial see 5.4.5.7.2	10 min / 25 min or field trial as claimed	as claimed	clean /dirty	2/4 or field trial see 5.4.5.7.2
other organisms	If claimed – 2,1 test modified			as claimed		According to test
other organisms	If claimed – simulated-use test or field trial					

NOTES on TABLES

[1] Requirements: basic requirements are mandatory and have to be fulfilled for authorisation of a product with this intended use. In addition, other organisms need to be tested, if claimed. If the requirements for these organisms are not fulfilled these organisms will be excluded from the claim.

[2] EN-tests are strongly advised but not mandatory. Other tests carried out according to standard guidelines are acceptable if a clear description of the test procedure (including contact time, soiling, temperature, suitable controls, lg reduction, etc.) and justification is provided. For information on recommended test strains please refer to Appendix 3 of this guidance.

[3] Contact time: maximum acceptable contact times at which efficacy should be demonstrated are stated. If a shorter contact time is stated on the label, efficacy has to be demonstrated at this shorter contact time. It is recommended to only use contact times mentioned in the EN standards as obligatory or additional contact time, to keep the robustness of the test as much as possible. When "as claimed" is stated as the contact time, the minimum contact time needs to respect that given in the respective EN standard. If longer contact time than that in the respective EN standard is claimed, a justification should be provided.

[4] It is recommended to use the temperatures of the EN standards when possible; adaptations may, however, be required to represent the use conditions. When the product is intended to be used at high temperatures (>40°C) temperature-tolerant test organisms should be used if efficacy tests with standard organisms are not valid anymore. See section **Error! Reference source not found.** of this guidance (sub-section "Temperature").

PT 2 hard surfaces and other uses where EN tests are applicable, use in healthcare, and room disinfection / automated airborne disinfection of surfaces (including use in healthcare): according to EN standards additional temperatures (other than 20°C) may be allowed depending on the area of use.

PT 2 instrument disinfection: the test temperature should be adapted to 20-70°C in EN 14348, EN 14561, EN 14562, and EN 14563.

PT 3 hard surfaces and room disinfection/automated airborne disinfection of surfaces: for some uses, temperatures lower or higher than 10°C are relevant and should be tested.

PT 4 hard surfaces: food and feed area disinfectants are generally used at room temperature (test temperature 20°C), but for some uses and claims lower temperatures (e.g. surfaces in cold storage rooms), or higher temperatures (e.g. incubation rooms, warewash disinfection) are relevant and should be tested.

PT 4 inner surfaces without circulation/with CIP: The test temperature should be according to the use instructions on the label.

[5] Soiling conditions: Clean conditions are conditions representative of surfaces which have been cleaned satisfactorily and/or are known to contain minimal levels of organic and/or inorganic substances.

PT 1 and 2 hospitals and healthcare: Dirty 3 g/l bovine albumin + 3 ml/l sheep erythrocytes // Clean 0.3 g/l bovine albumin
PT 1 and 2 other uses: Dirty 3 g/l bovine albumin // Clean 0.3 g/l bovine albumin
PT 2 cosmetic industry: Dirty 3 g/l bovine albumin or 5 g/l sodium dodecyl sulfate // Clean 0.3 g/l bovine albumin

PT 2 surfaces in agricultural area (no plant protection claim): Dirty 10 g/l bovine albumin + 10 g/l yeast extract // Clean 3 g/l bovine albumin

PT 2 textiles The interfering substance most appropriate for the in-use conditions should be used

PT 3 general hard surface disinfectants, animal skin disinfection, pre-milking teat disinfection, and eggs in hatcheries: Dirty 10 g/l bovine albumin + 10 g/l yeast extract // Clean 3 g/l bovine albumin

PT 3 outer surfaces of milking equipment: Clean/Dirty 10 g/l skimmed milk

PT 3 teat disinfection:
pre milking: Dirty 10 g/l bovine albumin + 10 g/l yeast extract // Clean 3 g/l bovine albumin (different from EN 14885);
post milking: Clean/Dirty 10 g/l skimmed milk

PT3 textiles: depending on the use, either milk soiling (see teat disinfection) or veterinary soiling (see PT 3 general) would be the relevant type of soiling. However, since the phase 2, step 2 test for textile are not validated for this type of soiling, consultation with CEN is needed. For the time being, it is recommended to use the obligatory interfering substance in EN 16616: sterile defibrinated sheep blood (12.5 ml sheep blood per kg textile)

PT 4 general disinfection in food industry and other areas with surfaces in contact with food: Dirty 3 g/l bovine albumin // Clean 0.3 g/l bovine albumin

PT 4 milk industry and milking equipment on farms: Clean/dirty 10 g/l skimmed milk

PT 4 meat industry: Dirty 3 g/l bovine albumin // Slaughterhouses and other processes with blood: 3 g/l bovine albumin + 3 ml/l sheep erythrocytes // Clean 0.3 g/l bovine albumin

PT 5 general: Dirty ≥ 15 mg DOC/l // Clean $\underline{\geq}$ 2 mg DOC/l. To realize this DOC (Dissolved Organic Carbon) either yeast extract or BSA can be used: use of other interfering substances should be justified. The DOC should be measured before adding the test product, and adjusted with yeast extract or BSA to reach the required DOC/L
For EN 13623 the standard soiling of the test can be used or the test can be adapted with the soiling stated above.
For simulated-use test according to test protocol[30] clean conditions (2 mg DOC/l) are used, achieved from natural water, as described in the protocol.

PT 5 drinking water suppliers and water distribution system: primary disinfection: Dirty 15 mg DOC/l // Clean 2 mg DOC/l; secondary disinfection: Clean 2 mg DOC/l

PT 5 drinking water in reservoirs: origin raw water: Dirty 15 mg DOC/l // origin from drinking water supplier only: Clean 2 mg DOC/l

PT 5 drinking water for animals: origin raw water: Dirty 15 mg DOC/l // origin from drinking water supplier only: Clean 2 mg DOC/l

[6] According to EN 14885, activity on fungal spores is not regarded relevant for hygienic handrub and hygienic handwash products. Similarly activity on fungal spores and viruses is not regarded necessary for surgical handrub and surgical handwash, which are predominantly used to reduce the number of resident flora, which does not include those microorganisms.

[7] The first test is for medical applications and the second for non-medical applications. In case both types of applications are claimed, only one test has to be carried out, in which the relevant worst-case test conditions (in general medical test) are included.

[8] For hygienic handwash and handrub products used in medical area the contact time is usually 30 seconds for bactericidal and yeasticidal activity and virucidal activity against enveloped viruses. Please note that some EN tests (e.g. EN 14348) were not developed for hand disinfection and therefore contact times should be adapted according to the ones described in EN 13727 and EN 13624.

[9] According to EN 1500 the test is passed when the mean reduction achieved by the hygienic handrub product under test is at least not inferior to that achieved by a reference handrub with propan-2-ol 60 % (v/v) (p=0.025) tested with 60 s contact time.

[10] Different levels of virucidal activity: Virucidal activity against enveloped viruses/Limited spectrum virucidal activity/Virucidal activity. More information on these virucidal activity levels in section 5.4.1.2.2 (PT 1) and section 5.4.2.2.4 (PT 2 hard surface disinfection) of this guidance.

[11] For hygienic handwash products it is assumed that hands will not be washed before washing with a disinfectant. Therefore, tests have to be done under dirty conditions.

[12] For hygienic handwash products a concentration of 50% or lower has to be tested in EN 14348 and EN 14476 (as instructed in EN 13727, EN 13624, EN 1276 and EN 1650). The lower lg reductions only apply for hygienic handwash products used on wetted hands. When the products are applied on dry hands the higher lg reductions indicated in the standards apply.

[13] According to EN 1499 the test is passed when the mean reduction achieved by the hygienic handwash with the product under test is larger than that achieved by a specified reference hygienic handwash (unmedicated liquid soap) (p=0.01) tested with 60 s contact time.

[14] The WHO states that for several products, scrubbing for 2-3 minutes reduces bacterial counts to acceptable levels. However, in the past, longer scrubbing times were accepted. Contact times of longer than 3 minutes, and up to 5 minutes, will only be authorised with a sound justification on the necessity of such long scrubbing times. Shorter contact times are accepted when tested at this contact time.

[15] According to EN 12791 the test is passed when the mean reduction achieved by the surgical handrub product under test is at least not inferior to that achieved by a reference handrub with propan-1-ol 60 % (v/v) tested with 3 min contact time.

[16] Healthcare is defined as areas where disinfection or antisepsis is medically indicated. Such indications occur in patient care: e.g. in hospitals, in community medical facilities and dental institutions; in clinics of schools, of kindergartens and of nursing homes; and may also occur in the workplace and in the home. It may also include services such as in laundries and kitchens supplying products directly for the patient. Also, veterinary healthcare facilities are included.

[17] As long as no modifications are made to the DVG guideline to include EN soiling, the soiling (40% bovine serum) is regarded as dirty conditions.

[18] A CEN norm is under development and may be used.

[19] When a surface disinfectant is a ready-to-use wipe or a wipe, mop soaked with disinfectant liquid the product should be tested in the phase 2, step 2 test, with mechanical action (e.g. EN 16615). For more details see Table 8.

[20] For PT 2, other use than in healthcare: EN 16777 with Adenovirus and Murine Norovirus may be used. As soon as a phase 2, step 2 test for non-medical area is available, it should be used. For PT 4: either a modified EN 16777 with Murine Norovirus or, as soon as available, an EN food area test should be used. PT 2 and PT 4: for adapting soiling in EN 16777 please refer to footnote 4.

[21] Where it is not possible to test the product in a suspension test, the simulated-use test (phase 2, step 2) will be sufficient.

[22] EN 16616 should be used for biocidal products used in washing machines for all target organisms for which the test is validated. As soon as a suitable phase 2, step 2 test for viruses is available, that should be used. For products not intended to be used in washing machines, small scale laboratory setting (e.g. for pre-soaking in a bucket) may be considered (e.g. ASTM E2406 or ASTM E2274).

[23] For surface disinfection in veterinary areas the normal contact time is 5 min. For surface disinfection on the outside of animal transport vehicles (specifically tyres) the contact time should not exceed 5 min. For disinfectants used on boots applied by spraying or walk-through bath the contact time should not exceed 1 min.

[24] DVG guidelines are available at http://www.desinfektion-dvg.de/index.php?id=2219. Endoparasites: the pass criteria are for parasitic protozoans $\geq$95% reduction (*Cryptosporidium parvum*, phase 2, step 1 and phase 2, step 2 test) and for helminth eggs $\geq$98% (*Ascaris suum* phase 2, step 1) and $\geq$95% (*A. suum* phase 2, step 2).

[25] As soon as an EN standard phase 2, step 2 test is available this should be used.

[26] For pre-milking teat disinfection the normal contact time is 10-30 seconds. The maximum contact time is 60 seconds. For post-milking teat disinfection the normal contact time is 1 min. The maximum contact time is 5 min.

[27] For hoof disinfection it is not anticipated that hoofs will be cleaned sufficiently before disinfection in practice. Therefore only tests under dirty conditions are acceptable.

[28] For uses where efficacy against bacteriophages only is claimed, EN 13610 can be employed.

[29] UBA method "Quantitative determination of the efficacy of drinking water disinfectants", see Appendix 2 Table 41.

[30] E.g. according to CSTB (Centre Scientifique et Technique du Bâtiment) method. More information: Development of a pilot-scale 1 for *Legionella* elimination in biofilm in hot water network: heat shock treatment evaluation (Farhal et al. 2010, J. Appl. Microbiol. 1085:1073-1082); Chemical disinfection of *Legionella* in hot water system biofilm: A pilot scale 1 study (Farhal et al. 2011, Water Sci. Tech. 64:708-714, or according to UBA method presented in footnote 29.

[31] For reservoir water for animals the contact time should be as claimed.

Appendix 5. Examples of viruses sorted according to their presence in the human body in case of virus infection

These viruses may contaminate hands, instruments, other surfaces and textiles.

NOTE 1 This list is not exhaustive.
NOTE 2 Enveloped viruses are in **bold**.

Table 43: Examples of viruses

Blood	
Enterovirus	**Hepatitis C virus (HCV)**
Filoviridae	**Hepatitis Delta virus (HDV)**
Flavivirus	**Human Immunodeficiency Virus (HIV)**
Herpesviridae	**Human T Cell Leukaemia Virus (HTLV)**
Hepatitis A Virus (HAV)	Parvovirus B 19
Hepatitis B virus (HBV)	
Respiratory tract	
Adenovirus (Mast-)	**Influenza Virus**
Coronavirus	**Paramyxoviridae**
Enterovirus	Rhinovirus
Herpesviridae	**Rubella Virus**
Neuronal tissue, ear, nose and eyes	
Adenovirus (Mast-)	**Human Immunodeficiency Virus (HIV)**
Enterovirus	Polyomavirus
Herpesviridae	**Rabies Virus**
Measles Virus	**Rubella Virus**
Gastro-intestinal	
Adenovirus(Mast-)	Enterovirus
Caliciviridae	Hepatitis A Virus (HAV)
Coronavirus	Hepatitis E Virus (HEV)
Astrovirus	Rotavirus
Skin, breast and/or milk	
Enterovirus	**Human T Cell Leukaemia Virus (HTLV)**
Herpesviridae	Papillomavirus
Human Immunodeficiency Virus (HIV)	**Poxviridae**
Spleen and lymph nodes (see also blood)	
Human T Cell Leukaemia Virus (HTLV)	
Human Immunodeficiency Virus (HIV)	
Dental procedure	
Adenovirus(Mast-)	**Hepatitis C Virus (HCV)**
Enterovirus	**Hepatitis Delta Virus (HDV)**
Herpesviridae	**Human Immunodeficiency Virus (HIV)**
Hepatitis B virus (HBV)	
Urogenital tract	
Hepatitis B Virus (HBV)	**Human T Cell Leukaemia Virus (HTLV)**
Herpesviridae	Papillomavirus
Human Immunodeficiency Virus (HIV)	Polyomavirus

Reference:
Van Regenmortel MHV et al.,Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses. Academic Press, San Diego, 2000

Appendix 6. Selection of recommended tests for solid materials (excluding wood-preservatives)[60]

Table 44: Selection of recommended tests for solid materials (excluding wood-preservatives)

Standard Method + section reference	Title	Description	Possible application area
ISO 22196, Section 5.4.2.2	Measurement of antibacterial activity on plastics and other non-porous surfaces	Test to measure inhibition of bacterial growth on plastic material used in wet or humid conditions.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by inhibition of bacterial growth.
Section 5.4.2.3, Figure 4	Simulated Splash Model Non-Porous Materials	Test to measure killing on contact for non-porous material when the contaminant is spread by splashes. Speed of required effect (5-60 min) depends on claim.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by killing on contact to prevent cross-contamination
Section 5.4.2.3, Figure 5	Simulated Splash Model Porous Materials	Test to measure killing on contact for porous material when the contaminant is spread by splashes. Speed of required effect (5-60 min) depends on claim.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by killing on contact to prevent cross-contamination
Section 5.4.2.3, Figure 6	Printing Model	Test to measure killing on contact for non-porous material when the contaminant is spread by e.g. hand-contact. Speed of required effect (5-60 min) depends on claim.	Treated articles in PT 2, 3, 4, with a claim to protect people/animals by killing on contact to prevent cross-contamination
BS 3900 Part G6, Section 5.5.8.1	Methods of test for paints. Part G6: Assessment of resistance to fungal growth	Painted panels inoculated with a mixture of spores of fungi known to colonise paints exposed to humid conditions for up to 12 weeks should show visual appearance of fungal growth. The treated sample should be free of it.	PT 7
ASTM G21-09, Section 5.5.8.2	Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi	The synthetic polymer portion of plastic materials is usually fungus-resistant in that it does not serve as a carbon source for the growth of fungi. It is generally the other components, such as plasticizers, cellulosics, lubricants, stabilizers, and	PT 7, 9

[60] These tests are not necessarily appropriate for all claims and materials. Tests have to be chosen depending on the claim made, the materials used and the conditions of use foreseen for the treated material/article.

Standard Method + section reference	Title	Description	Possible application area
		colorants, that are responsible for fungus attack on plastic materials.	
ISO 846: 1997, Section 5.5.8.2	Plastics - Evaluation of the action of microorganisms	Method for determining the deterioration of plastics due to the action of fungi and soil microorganisms by visual appearance, changes in mass or changes in physical properties. The aim is not to determine the biodegradability of plastics. Includes even a soil burial variant. Note: the section covering bacteria is not considered to be useful.	PT 7, 9
ISO 16869:2008, Section 5.5.8.2	Plastics - Assessment of the effectiveness of fungistatic compounds in plastics formulations	Method for determining the effectiveness of fungistatic compounds in protecting susceptible ingredients like plasticizers, stabilizers, etc., in plastics formulations. A minimum diffusion of the fungicide out of the matrix is necessary as the spores are added in an agar-layer. Evaluation by visual examination.	PT 7, 9
BS EN 60068-2-10:2005, Section 5.5.8.1	Environmental testing. Tests. Test J and guidance: Mold growth	Test for fungal and microbial resistance applicable to a wider range of materials	PT 7, 9
OECD (OECD ENV/JM/MONO(2014)18 Section 5.5.8.5.2	Guidance Document for Quantitative Method for Evaluating Antibacterial Activity of Porous and Non-Porous Antibacterial Treated Materials.	Method for measuring the inhibition of bacterial growth or metabolism of porous and non-porous materials that have been treated with a biocide.	Anti-odour testing for textiles, PT 9
IBRG TEX13-005.4, Section 5.5.8.5.2	Tier 1 Textile Method Antibacterial Properties	Method to determine the basic antibacterial properties of textiles and porous materials and articles treated with a biocide.	Anti-odour testing for textiles, PT 9

Appendix 7. Selection of recommended tests for liquid materials[61]

Table 45: Selection of recommended tests for liquid materials

Reference + section reference	Title	Description	Possible application area
IBRG P 16-001.2, Section 5.5.7	Tier 1 Wet State Paint Method	A Method for Determining the Basic Efficacy of Biocidal Active Substances in aqueous based paints.	PT 6
IBRG PDG 16-001.2, Section 5.5.7	Tier 1 Polymer dispersion Method	A Method for Determining the Basic Efficacy of Biocidal Active Substances used in polymer dispersions.	PT 6
IBRG PDG 16-007.2, Section 5.5.7	Tier 1 Basic Efficacy Method for Biocidal Active Substances used to Preserve Aqueous-Based Products	Method for determining the basic efficacy of biocidal active substances for in-can preservation in aqueous based products	PT 6
IBRG FFG 16-001.4, Section 5.5.13	Tier 1 Metal Working Fluids Method	Method for determining the basic efficacy of biocidal active substances in aqueous based metalworking fluids.	PT 13

[61] These tests are not necessarily appropriate for all claims and materials. Tests have to be chosen depending on the claim made, the materials used and the conditions of use foreseen for the treated material/article.

Appendix 8. Commonly Used Methods to Measure the Effects of Preservative/Curative Action in Liquid Matrices[62]

Table 46: Commonly Used Methods to Measure the Effects of Preservative/Curative Action in Liquid Matrices

Reference	Title	Description	PT
ASTM D2574-06	Standard Test Method for Resistance of Emulsion Paints in the Container to Attack by Microorganisms	This test method covers the determination of the relative resistance of emulsion paints to attack in the container by microorganisms.	PT 6
ASTM D4783-01e1	Standard Test Methods for Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi	Determination of the resistance of liquid adhesive preparations to microbial attack in the container by challenging adhesive specimens with cultures of bacteria, yeasts, or fungi, and checking for their ability to return to sterility. These test methods return qualitative results.	PT 6
ASTM E1259-05	Standard Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C	The procedure should be used to evaluate the relative efficacy of microbicides in liquid fuels boiling below 390°C. The effect of environmental conditions, such as a variety of fuel additives, metal surfaces, and climatology, are variables that can be included in specific tests using this protocol.	PT 6
SABS 1102 (1987)	Bacterial efficacy of biocides used in water-based emulsion paints	Efficacy test for in can preservatives in paints (emulsion) against bacteria.	PT 6
NF X41-520 March 1968	Protection. Testing method for resistance of paints to microorganisms and their protective power.		PT 6
ASTM E645:2018	Standard Practice for Evaluation of Microbicides Used in Cooling Water Systems	This method is designed to evaluate the efficacy of microbicides (algaecides, bactericides, and fungicide) used for controlling microbial growth in cooling water systems, for the preservation of processing liquids, and for the treatment of raw waters for papermaking processing.	PT 11/12
EN 13623:2020	Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity against *Legionella* of chemical disinfectants for aqueous systems - Test method and requirements (phase 2, step 1)	This European Standard specifies a suspension method for testing bactericidal activity against *Legionella* by assessing reduction in the number of viable *Legionella* cells in suspension under defined conditions. The approach can be applied to formulated products or to biocidal active substances.	PT 11

[62] Please note: The methods listed are not necessarily appropriate in all cases. Their applicability depends on the claim made, the materials used and the conditions of use for the treated material/article. These methods are listed to give an overview for the assessor when and where a method is meaningful to demonstrate a claim and where its limits are.

Reference	Title	Description	PT
ASTM E1839:2020	Standard Test Method for Efficacy of Slimicides for the Paper Industry--Bacterial and Fungal Slime	This test method presents a procedure to evaluate the efficacy of slimicides for the control of bacterial and fungal slime in paper mill systems and their counterparts.	PT 12
ASTM E723:2019	Standard Practice for Evaluation of Antimicrobials as Preservatives for Aqueous-Based Products Used in the Paper Industry (Bacterial Spoilage)	This laboratory practice is used to determine the efficacy of an antimicrobial for preventing bacterial spoilage of in-process aqueous-based products used in the paper industry.	PT 12
ASTM E875:2017	Standard Test Method for Efficacy of Fungal Control Agents as Preservatives for Aqueous-Based Products Used in the Paper Industry	This laboratory test method is used to determine the efficacy of a fungal control agent to prevent spoilage of in-process aqueous-based products used in the paper industry.	PT 12
NACE TM0194-2014	Standard Test Method - Field monitoring of bacterial growth in oil and gas systems	This standard give recommendation for field monitoring method in oil and gas systems.	PT 12
IBRG FFG 19.006-02	Tier 1 Basic Efficacy of Biocidal Active Substances used in Aqueous-Based Cooling Fluid	This method is designed to evaluate the preventive efficacy of bactericide and fungicide product for preservation of cooling waters. This method can also be adapted for the preservation of processing liquids or to demonstrate preventive efficacy of algaecide.	PT 11
IBRG FFG 19.007-02	Tier 1 Basic Efficacy of Biocidal Active Substances used to Prevent Biofilms in Aqueous-Based Cooling Fluid	This method is designed to evaluate the preventive efficacy on biofilm formation in aqueous-based cooling fluids. This method can be adapted to evaluate the preventive efficacy on biofilm formation in processing liquids.	PT 11
IBRG FFG 19.008-02	Tier 1 Basic Efficacy of Biocidal of Biocidal Active Substances used as Slimicides in Aqueous-Based Paper Pulps	This method is designed to evaluate the preventive efficacy of slimicides for preservation of paper pulps and similar fluids in the paper process.	PT 12
IBRG FFG 21/010/01	Efficacy of Products used as Preservatives of Fluids Used in the Oil and Gas Extraction Industries - Anaerobic Bacterial Biofilms Preservative	This document describes a method for determining the basic efficacy of biocidal active substances in fluids used in the oil and gas extraction industries	PT 12
IBRG FFG 21/011/01	A Method for Determining the Basic Efficacy of Biocidal Active Substances used as Curative Agents against Anaerobic Planktonic Bacterial Populations and Biofilms in Aqueous-Based Systems	This document describes a method for determining the basic efficacy of biocidal active substances in aqueous based fluids.	PT 12
ASTM E2275-03e1 (replaces D3946 and E686)	Standard Practice for Evaluating Water-Miscible Metalworking Fluid. Bioresistance and Antimicrobial Pesticide Performance	Laboratory procedures for rating the relative inherent bioresistance of water-miscible metalworking fluids, the bioresistance attributable to augmentation with antimicrobial pesticides or both, for	13

Reference	Title	Description	PT
		determining the need for microbicide addition prior to or during fluid use in metalworking systems and for evaluating microbicide performance. Relative bioresistance is determined by challenging metalworking fluids with a biological inoculum that may either be characterized (comprised of one or more known biological cultures) or uncharacterized (comprised of biologically contaminated metalworking fluid or one or more unidentified isolates from deteriorated metalworking fluid). Challenged fluid bioresistance is defined in terms of resistance to biomass increase, viable cell recovery increase, chemical property change, physical property change or some combination thereof. This practice is applicable to antimicrobial agents that are incorporated into either the metalworking fluid concentrate or end-use dilution. It is also applicable to metalworking fluids that are formulated using non-microbicidal, inherently bioresistant components. The results of tests completed in accordance with this practice should be used only to compare the relative performance of products or microbicide treatments included in a test series. Results should not be construed as predicting actual field performance.	
ASTM E979-91(2004)	Standard Test Method for Evaluation of Antimicrobial Agents as Preservatives for Invert Emulsion and Other Water Containing Hydraulic Fluids	This laboratory test method is designed to utility and effectiveness of antimicrobial ag to control microbial growth in invert emulsi water containing hydraulic fluids.	PT 13
ASTM WK8252	New Standard Test Method for Determining Resistance of Aqueous Metalworking Fluids towards Non-Tuberculous, Environmental Mycobacteria	Determines the relative bioresistance of aqueous metalworking fluids towards non-tuberculous (NTM), rapidly growing (RGM), environmental mycobacteria by challenging them with a mycobacterial inoculum isolated from actual spoiled metalworking fluid field samples from the user/s site. In order to simulate field conditions, another challenge inoculum consisting of a mixture of common metalworking fluid spoilage microorganisms originating from actual MWF field samples is also used	PT 13
SABS 1435-1987	South African standard specification for biocides for use in emulsions of aqueous metal working fluid and aqueous hydraulic fluid.		PT 13
Rawlinson and	A recirculating test rig for the investigation of metal-working fluid	The method described, which attempts to simulate the conditions under which a	PT 13

Reference	Title	Description	PT
Shennan, 1987.	spoilage. In Industrial microbiological testing 1987 pp. 227-231. Edited by Hopton and, J.W.; Hill, E.C.	metal working fluid will be used in service, has been used extensively for the testing of new product formulations and the evaluation of biocides.	
UK MOD 91-70 issue (1990)	Cutting fluid, soluble, biostable joint service designation ZX-9		PT 13

Appendix 9. Commonly Used Methods to Measure the Effects of Protecting Material[63]

Table I: Methods used to Examine the Resistance of Porous Materials to Biodeterioration: Textiles

Reference	Title	Description	Major Principle/Use
EN 14119:2003	Testing of textiles – Evaluation of the action of microfungi	The test is designed to determine the susceptibility of textiles to fungal growth. Assessment is by visual rating and measurement of tensile strength.	Agar plate test
AATCC 30-2004	Antifungal activity, Assessment on textile materials: mildew and rot resistance of textile materials	The two purposes of the test are to determine the susceptibility of textiles to microfungi and to evaluate the efficacy of fungicides on textiles.	Agar plate test
DIN 53931	Testing of textiles; determination of resistance of textiles to mildew; growth test	The test determines the efficacy of treatments for prevention of fungal growth on/in textiles. It also allows the performance testing of a treatment after UV irradiation , leaching etc.	Agar plate test
MIL-STD-810F	Environmental Engineering considerations and laboratory tests; Method 508.5 FUNGUS	The purpose of the method is to assess the extent to which a material will support fungal growth and how performance of that material is affected by such growth.	Humid chamber test (90 to 99% humidity)
BS 6085 :1992	Determination of the resistance of textiles to microbial deterioration	The purpose of the method is to assess the extent to which a material will support fungal/bacterial growth and how performance of the material is affected by such growth. Visual Assessment and measurement of tensile strength.	a) soil burial test; b) agar plate test, c) humid chamber test
EN ISO 11721-1 (2001)	Textiles - Determination of resistance of cellulose-containing textiles to micro-organisms: Soil burial test Part 1: Assessment of rot retarding finishing	The test is designed to determine the susceptibility of cellulose containing textiles against deterioration by soil micro-organisms. Preserved and unpreserved textiles are compared. Visual Assessment and measurement of tensile strength.	Soil burial test
EN ISO 11721-2 (2003)	Textiles - Determination of resistance of	The test identifies the long-term resistance of a rot-retardant finish against the attack of soil inhabiting micro-organisms. It allows to make a	Soil burial test

[63] Please note: The methods listed are not necessarily appropriate in all cases. Their applicability depends on the claim made, the materials used and the conditions of use for the treated material/article. These methods are listed to give an overview for the assessor when and where a method is meaningful to demonstrate a claim and where its limits are.

	cellulose-containing textiles to micro-organisms: Soil burial test Part 2: Identification of long-term resistance of a rot retardant finish	distinction between regular long-term resistance and increased long-term resistance. Visual Assessment and measurement of tensile strength	
BS 2011 : Part 2.1J (IEC 68-2-10)	Basic environmental testing procedures	Mould growth test to show the susceptibility of a material towards colonization by fungi.	Humid chamber test (90 to 99% humidity)
AS 1157.2 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Textiles to Fungal Growth. Section 1 - Resistance to Surface Mould Growth.	Test specimens are inoculated with a suspension of spores of *Aspergillus niger* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary. Specimens are examined for the presence of surface mould growth.	Agar plate test
AS 1157.4 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Textiles to Fungal Growth. Section 2 - Resistance to Cellulolytic Fungi.	Test specimens are inoculated with a suspension of spores of *Chaetomium globosum* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test
AS 1157.3 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Cordage and Yarns to Fungal Growth.	Test specimens are inoculated with a suspension of spores of *Chaetomium globosum* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test.	Agar plate test (other vessels containing media are employed for large specimens).

Table II: Methods used to Examine the Resistance to Biodeterioration: Geotextile

Reference	Title	Description	Major Principle
EN 12225:2000	Geotextiles and Geotextiles-related products - Method for determining the microbiological resistance by a soil burial test	The test is designed to determine the susceptibility of geotextiles and related products to deterioration by soil micro-organisms. Visual Assessment and measurement of tensile strength.	Soil burial test

Table III: Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Paper *etc.*			
Reference	**Title**	**Description**	**Major Principle**
DIN EN 1104 - 05	Paper and board intended to come into contact with foodstuffs Determination of transfer of antimicrobic constituents	A minimum of 20 replicate sub-samples (each 10 - 15 mm in diameter) taken from 10 samples of a batch of paper are placed in intimate contact with nutrient agar plates inoculated with either *Bacillus subtilis* or *Aspergillus niger* and incubated at 30° C for 7 days and at 25° C for 8 - 10 days respectively.	Zone Diffusion Assay.
ASTM D 2020-03	Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard - Direct Inoculation	Replicate samples (3) are inoculated with a suspension of fungal spores and then incubated on the surface of a minimal mineral-salts medium to determine if they support fungal growth.	Biodeterioration Test.
ASTM D 2020-03	Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard - Soil Burial	Replicate samples (5) are buried in soil for 14 days and then examined for the deterioration compared with unburied samples for both physical deterioration and loss of tensile strength.	Biodeterioration/ Biodegredadation Test.
AS 1157.7 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 6: Resistance of Papers and Paper Products to Fungal Growth.	Test specimens are placed on the surface of a mineral-salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Growth on the specimen is assessed.	Agar plate test
AS 1157.5 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 5: Resistance of Timber to Fungal Growth.	Test specimens are placed on the surface of a mineral salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Growth on the specimen is assessed.	Agar plate test
AS 1157.6 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 6: Resistance of Leather and Wet 'Blue' Hides to Fungal Growth.	Test specimens are placed on the surface of a mineral salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Growth on specimens is assessed. Sucrose containing media is employed where true controls cannot be obtained.	Agar plate test

Table IV: Methods used to Examine the Resistance to Biodeterioration: Plastics			
Reference	**Title**	**Description**	**Major Principle**
ASTM D 5338 - 92	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Biodegradability test
ASTM E 1428 - 99	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Agar plate test
ASTM G 22 - 76	Agar plate test	Agar plate test	Agar plate test
ASTM G 21 - 96	Agar plate test	Agar plate test	Agar plate test
ASTM G 29 - 96	Agar plate test	Agar plate test	Biofouling test
EN 14047:2002	Agar plate test	Agar plate test	Biodegradability test
EN 14048:2002	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Biodegradability test
ISO 846:1997	Humid chamber test (90 to 99% humidity)	Humid chamber test (90 to 99% humidity)	Agar plate test; soil burial test
EUROCAE ED-14B/ RTCA DO 160B	Agar plate test	Agar plate test	Humid chamber test (90 to 99% humidity)
MIL-STD-810F	Environmental Engineering considerations and laboratory tests; Method 508.5 FUNGUS	The purpose of the method is to assess the extent to which a material will support fungal growth and how performance of the material is affected by such growth.	Humid chamber test (90 to 99% humidity)
BS 2011 : Part 2.1J (identical with IEC 68-2-10)	Basic environmental testing procedures	Mould growth test to show the susceptibility of a material towards the colonization by fungi.	Humid chamber test (90 to 99% humidity)
ISO 16869:2008	Plastics - Assessment of the effectiveness of fungistatic compounds in plastics formulations	A specimen is placed on a nutrient-salt- agar (without additional carbon source) in a petri dish and overlayed with the same agar containing fungal spores. Rate of growth on the specimen is visually assessed.	Agar plate test
AS 1157.4 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 4: Resistance of Coated Fabrics and	Test specimens are inoculated with a suspension of spores of *Chaetomium globosum* and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test. Glass	Agar plate test

	Electronic Boards to Fungal Growth.	rings are employed to hold the specimens in intimate contact with agar when necessary.	
AS 1157.11 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 11: Resistance of Rubbers and Plastics to Surface Fungal Growth - Section 1: Resistance to Growth	Test specimens are inoculated with a suspension of spores of a range of fungi and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test
AS 1157.11 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 11: Resistance of Rubbers and Plastics to Surface Fungal Growth - Section 2: Fungistatic Properties	Test specimens are placed on the surface of a sucrose, mineral salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary. Growth on both the specimen and inhibition of growth on the surrounding agar are assessed.	Agar plate test

Table V: Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Surface Coatings & Adhesives

Reference	Title	Description	Major Principle
BS3900 Part G6	Assessment of resistance to fungal growth	Replicate test panels coated with the test coating are inoculate with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under conditions suitable to support fungal growth (23 ± 2°C and high humidity/surface condensation). In the published standard, condensation on the test panels is achieved by increasing the temperature in a water bath below the samples for short periods of time. Revisions are in progress which may obviate this step. The method is validated if fungal growth/germination of spores is observed after two weeks on a standard coating known to be susceptible to fungal growth. After incubation growth is rated in accordance with a scale related to the percent cover with fungal growth (following visual and microscopical examination). A natural and artificial soiling are described in the method which can be employed when appropriate.	Biodeterioration Test
ASTM D3273-12	Standard Test Method for Resistance to Growth of Mold on the	Replicate test panels coated with the test coating are inoculated with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are	Biodeterioration Test

	Surface of Interior Coatings in an Environmental Chamber	then incubated under conditions suitable to support fungal growth.	
WK4201	Standard Test Method for Resistance to Mold Growth on Building Products in an Environmental Chamber	Replicate test panels coated with the test coating are inoculated with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under conditions suitable to support fungal growth.	Biodeterioration Test
ASTM D5590-94	Standard Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay		Agar Plate Test
SS345 Appendix 9	Formal Title Missing at Present	The bottom of glass petri dishes are coated with paint. After drying, a culture of algae in a suitable growth liquid medium is placed into the dish and incubated under conditions suitable for algal growth.	Biodeterioration Test.
EN 15457:2007	Paints and varnishes – Laboratory method for testing the efficacy of film preservatives in a coating against fungi	Coatings are applied to glass fibre discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of spores of 4 fungal species selected from a list of 10. The plates are then incubated at 24°C for 21 days and then assessed for growth using a rating scale. The test is intended to support claims that a biocide can have an effect in a surface coating in support of its listing in the relevant use category within the EU BPD. It is not intended to assess the performance of surface coatings.	Zone Diffusion Assay
AS 1157.10 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 10: Resistance of Dried or Cured Adhesives to Fungal Growth	Test materials coated onto glass microscope slides are inoculated with a suspension of spores of a range of fungal species and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth.	Agar plate test
EN 15458:2007	Paints and varnishes – Laboratory method for testing the efficacy of film preservatives in a coating against algae	Coatings are applied to glass fibre discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of 3 algal species selected from a list of 5. The plates are then incubated at 23°C under illumination (16 hour day length, 1000 Lux) for 35 days and then assessed for	Zone Diffusion Assay

		growth using a rating scale. The test is intended to support claims that a biocide can have an effect in a surface coating in support of its listing in the relevant use category within the EU BPD. It is not intended to assess the performance of surface coatings.	
VdL RL06	Guideline to Evaluate the Resistance of Coating Materials against Mold Growth	Coatings are applied to paper discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of spores of *A niger* and *Penicillium funiculosum*. The plates are then incubated at 28°C for 3 weeks and assessed for growth using a rating scale after 1, 2 and 3 weeks. Coatings for exterior use and 'wet' applications are leached in water prior to testing.	Zone Diffusion Assay/Humid Chamber Test
VdL RL07	Guideline to Evaluate the Resistance of Coating Materials against Mold Growth	Coatings are applied to paper discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of *Scenedesmus vacuolaris and Stichococcus bacillaris*. The plates are then incubated at 23°C for 3 weeks under illumination (16 hour day length, 1000 Lux) and assessed for growth using a rating scale after 1, 2 and 3 weeks. Coatings for exterior use and 'wet' applications are leached in water prior to testing.	Zone Diffusion Assay/Humid Chamber Test

Table VI: Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile/wadding)

Reference	Title	Description	Major Principle
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Qualitative Test	Three replicate samples of fabric, yarn or pile/wadding are placed in intimate contact with the surface of agar plates that have been inoculated with a cell suspension of either *Staphylococcus aureus* or *Klebsiella pneumoniae* and incubated at 37° C for 24 - 48 hours. The presence of and size of any zone of inhibition around the samples is then recorded.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Quantitative Test	Replicate samples of fabric (6 of the control and 3 of the treated) are inoculated with individual bacterial species (*e.g. S. aureus* and *K. pneumoniae*) suspended in a heavily diluted nutrient medium. The samples are incubated under humid conditions at 37° C for a specified contact time. Activity is	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		assessed by comparing the size of the initial population in the control with that present following incubation. No neutraliser is employed during cell recovery.	
EN ISO 20645 - 2004	Textile Fabrics - Determination of the antibacterial activity - Agar plate test (ISO/FDIS 20645:2004)	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *S. aureus*, *Escherichia coli* or *K. pneumoniae*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed for growth based on either the presence of a zone of inhibition of > 1 mm or the absence/strength of the growth in the media overlaying the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN 195920	Examination of the Antibacterial Effect of Impregnated Textiles by the Agar Diffusion Method	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *S. aureus* or *E. coli*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed as described in BS EN ISO 20645 above.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN195924	Textile Fabrics - Determination of the Antibacterial Activity: Colony Plate Count Method	Fifteen replicate samples (each replicate is comprised of sufficient specimens of 25 ± 5 mm to absorb 1 ml of test inoculum) are inoculated with cells of either *E. coli* or *S. aureus* suspended in a liquid nutrient medium and incubated in sealed bottles for up to 24 hours at 27° C. After 0, 6 and 24 hours, 5 replicate samples are analysed for the size of the viable population present. A neutraliser is employed. An increase of 2 orders of magnitude of the population exposed to a control sample is required to validate the test. The method defines a textile as antibacterial if no more than a specified minimum level of growth is observed after 24	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		hours in 4 of the 5 replicate groups of samples.	
SN195921	Textile Fabrics - Determination of Antimycotic Activity: Agar Diffusion Plate Test	Replicate (4) samples of sterilised fabric (25 ± 5 mm diameter) are placed in intimate contact with a solid nutrient medium in a petri dish. Each petri dish has been prepared as a double layer. The first layer consists of 10 ml nutrient agar, the second layer of another 10 ml of the same nutrient agar to which 0.1 ml spore suspension (10^7 ml^{-1}) of either *Candida albicans*, *Aspergillus niger*, *Cladosporium sphaerospermum* or *Trichophyton mentagrophytes* had been added. The plates are then incubated at 28° C either 2 days (*C. albicans*) or 7 days (*A. niger*, *C. sphaerospermum* and *T. mentagrophytes*). The test is valid when control specimens of the same material without biocide, or of a biocide-free standard specified cotton material are fully overgrown. Good antimycotic efficacy is considered to be demonstrated when the specimens show no fungal growth on their surface. The test specifies that both sides of a material have to be tested.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Absorption method	Replicate (6) samples of textile are inoculated with a standardised broth culture of either *S. aureus* or *K. pneumoniae* in individual tubes and then incubated at 37° C for 18 - 24 hours in closed containers. Samples are analysed for the presence of viable bacteria both before and after incubation by either total viable count or the determination of total ATP. Samples are sterilised prior to testing and a neutraliser is employed during recovery. The test is validated by growth of ^1 order of magnitude during the incubation period.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Transfer method	Replicate (6) samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *S. aureus* and *K. pneumoniae* using a 200	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		g weight for 1 minute. The samples are then removed. Replicate (3) samples are analysed for either the number of viable bacteria or the total ATM content both before and after incubation under humid conditions at 37° C for 24 hours. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either growth of ^1 order of magnitude during the incubation period or by a measure of the variability of the data obtained.	
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Printing method	Replicate (6) samples of test material are either *S. aureus* and *K. pneumoniae* by 'printing' cells collected on a membrane filter onto their surface in a standardised manner. The samples are then incubated under humid conditions for 18 - 24 hours at 20° C for a specified contact time(s). Replicate (3) samples are analysed for either the number of viable bacteria or the total ATM content both before and after incubation. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either determining the survival of the inoculum on the control material.	'Dry' inoculum intimate contact test. The transfer method of inoculation could be adapted to provide some simulation data.
ISO/FDIS 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 1 - Luminescence Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the ATP concentration associated with the samples. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced concentrations of ATP associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.
ISO/WD 13629-1	Textiles - Determination of Antifungal Activity of Textile Products:	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an	Basic efficacy test that has limited use as a simulation of final use of a treated material.

	Part 2 - Plate Count Method	agar surface and then incubated. Germination and growth of the spores is followed by measuring the number of colony forming units. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced numbers of colony forming units associated with the treated material in comparison with the untreated material.	The transfer method of inoculation could be adapted to provide some simulation data.

Table VII: Methods used to Examine the Antimicrobial Activity of Carpets

Reference	Title	Description	Major Principle
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Qualitative Antibacterial Activity	Petri dishes with nutrient media are inoculated with a single, diagonal streak (approx.7.5 cm) of either *S. aureus* or *K. pneumoniae*. An unsterilized test specimen (25 mm x 50 mm) is placed in intimate contact and transversely across the inoculum on the agar surface. The plates are then inoculated at 37° C for 18 - 24 hours. The front and back of the carpet are tested separately. After incubation, the plates are inspected for the presence of growth both below the specimens and for any zone of inhibition surrounding the specimens. The test can also be used to test the effect of cleaning regimes. An untreated control is optional.	Qualitative assessment of rate of kill and zone diffusion test Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antibacterial Activity	Unsterilized specimens of carpet are pre-wetted with either sterile water or a wetting agent before being inoculated with individual suspensions of either *S. aureus* or *K. pneumoniae* in either a low or a high nutrient solution. The samples are then incubated in a tightly closed jar at 37° C for a specified contact time. Cells are recovered in 100 ml of a neutraliser after 0 and 6 - 24 hours of incubation. Activity is assessed by comparing the size of the initial population in the control (if used) with that present following incubation. A control is optional. When not	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		employed, viable counts following incubation of the treated specimens alone are considered. The test can also be used to test the effect of cleaning regimes.	
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antifungal Activity	Petri dishes containing Sabouraud Dextrose Agar are inoculated with 1 ml of a spore suspension of *Aspergillus niger*. Immediately afterwards, specimens (38 mm diameter) of unsterile test material are placed into intimate contact with the agar. An additional 0.2 ml of the same spore suspension is also employed to inoculate the test pieces directly. The samples are then incubated at 28°C for 7 days. The back and front of the discs of carpet are tested in separate dishes. The zone of inhibition and the growth of fungus on the upper surface of the specimens are reported (no growth, microscopic growth, macroscopic growth). The test can also be used to test the effect of cleaning regimes.	Zone diffusion test/surface growth test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
WIRA Test F	Test Method for Assessing the Survival of Test Organisms on Floor Coverings	Specimens (850 mm x 350 mm) are conditioned at 20°C and 65% RH before being subjected to 2 wet and 2 dry passes using a commercial spray extraction machine or a test rig. After 24 h drying, 12 specimens (each 60 mm diameter) are cut from the carpet. An aliquot (1 ml) of a suspension of cells of *E. coli* in nutrient broth is poured onto filter paper (7 cm diameter). The filter paper is then pressed for 1 min onto the surface of the carpet using a 1 kg weight. The filter paper is then discarded. After 0, 6 and 24 hours incubation at a specified temperature the carpet´s surface is pressed onto contact plates of McConkey agar. After 24h replicate (3) plugs (10 mm) are taken from each specimen and suspended in 10 ml nutrient broth for 30 seconds and then analysed for the presence of *E. coli* by total viable count.	Cell suspension intimate contact test. Potential to demonstrate the effectiveness of an antimicrobial treatment if appropriate incubation conditions are selected and addition species employed.

Table VIII: Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
JIS Z 2801: 2000	Antimicrobial products - Test for antibacterial activity and efficacy	The surface of replicate sample (3 for each treatment and 6 for the blank reference material - usually 50 mm x 50 mm) are inoculated with a suspension of either *E. coli* or *S. aureus* in a highly diluted nutrient broth. The cell suspension is then held in intimate contact with the surface by the use of a sterile polyethylene film (usually 40 mm x 40 mm) for 24 hours at 35° C under humid conditions. The size of the population on the treated surface is then compared with the size on the control surface both prior to and after incubation. A neutraliser for certain biocide types is employed. Antibacterial activity is certified if the difference between the lg_{10} of the population on the treated sample and that on the control surface is > 2.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 22196:2011	Plastics - Measurement of antibacterial activity on plastics surfaces.	This is the current New Work Proposal at ISO created from JIS Z 2801 by the SIAA of Japan. Modification and validation is in progress in collaboration with the IBRG. Some changes are expected.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité antibactérienne	Four replicate samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *S. aureus* and *K. pneumoniae* using a 200g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37°C for 24 hours. A neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ASTM E2180-07	Standard Test Method for Determining the Activity of Incorporated	Replicate (3) samples of material are inoculated with cells of either *S. aureus* or *K. pneumoniae* suspended in molten semi-solid isotonic	Immobilised cell suspension intimate contact test. Basic efficacy test that has limited use as a

	Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials	saline/agar. This attempts for form an 'artificial biofilm' which holds the suspension in intimate contact with the test surface of inherently hydrophobic materials. Samples are then incubated at a temperature similar to that intended for the final use for a specified period (usually 24 hours) under humid conditions. The size of the viable bacterial populations on the control and treated surfaces is then determined using a dilution plate count. Any effect is recorded using percent reduction calculated from the geometric means of the data. A neutraliser may be employed and sonication is used to separate the 'biofilm' from the test surfaces and suspend the agar gel. Subsequent imprinting of the test surface onto solid nutrient media can be performed to look for the presence of adherent viable cells.	simulation of final use of a treated material.
ASTM E2149-10	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of *Klebsiella pneumoniae* and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	Relies on either diffusion of antimicrobial agents from treated material into the cell suspension or due to interaction between the population and the surface of the material in suspension. Basic efficacy test that has limited use as a simulation of final use of a treated material.

Appendix 10. Commonly Used Methods to Measure Antimicrobial Activity[64]

Table VI: Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile/wadding)

Reference	Title	Description	Major Principle
ASTM E2149-10	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of *Klebsiella pneumoniae* and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	Relies on either diffusion of antimicrobial from treated material into the cell suspension. Some activity may be due to interaction between the population and the surface of the material in suspension. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 147-2011	Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method	Agar plates are inoculated with 5 parallel streaks (60 mm long) of either *Staphylococcus aureus* or *K. pneumoniae.* A textile sample is then placed over the streaks and in intimate contact with the surface of the agar and incubated. Activity is assessed based on either the mean zone of inhibition over the 5 streaks or the absence of growth behind the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 100-2012	Antibacterial Finishes on Textile Materials: Assessment of.	Replicate samples (sufficient to absorb 1 ml of test inoculum) of fabric are inoculated with individual bacterial species (*e.g. S. aureus* and *K. pneumoniae*) suspended in a nutrient medium. The samples are incubated under humid conditions at 37° C for a specified contact time. Activity is assessed by comparing the size of the initial population with that present following incubation. A neutraliser is employed recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes - Étoffes et	Four replicate samples of test material are placed in contact with	Cell suspension

[64] Please note: The methods listed are not necessarily appropriate in all cases. Their applicability depends on the claim made, the materials used and the conditions of use for the treated material/article. These methods are listed to give an overview for the assessor when and where a method is meaningful to demonstrate a claim and where its limits are.

	surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité antibactérienne	an agar plate that has been inoculated with a specified volume of a known cell suspension of either *S. aureus* and *K. pneumoniae* using a 200 g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37° C for 24 hours. A neutraliser is employed during cell recovery.	intimate contact test. The transfer method of inoculation could be adapted to provide some simulation data.
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Qualitative Test	Three replicate samples of fabric, yarn or pile/wadding are placed in intimate contact with the surface of agar plates that have been inoculated with a cell suspension of either *S. aureus* or *K. pneumoniae* and incubated at 37° C for 24 - 48 hours. The presence of and size of any zone of inhibition around the samples is then recorded.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Quantitative Test	Replicate samples of fabric (6 of the control and 3 of the treated) are inoculated with individual bacterial species (*e.g. S. aureus* and *K. pneumoniae*) suspended in a heavily diluted nutrient medium. The samples are incubated under humid conditions at 37° C for a specified contact time. Activity is assessed by comparing the size of the initial population in the control with that present following incubation. No neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
EN ISO 20645 - 2004	Textile Fabrics - Determination of the antibacterial activity - Agar plate test (ISO/FDIS 20645:2004)	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *S. aureus*, *Escherichia coli* or *K. pneumoniae*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed for growth based on either the presence of a zone of inhibition of > 1 mm or the absence/strength of the growth in the media overlaying the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN 195920	Examination of the Antibacterial Effect of Impregnated	Four replicate samples of fabric (25 ± 5 mm) are placed in intimate contact with a solid nutrient	Zone diffusion assay. Basic efficacy test that

	Textiles by the Agar Diffusion Method	medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either *S. aureus* or *E. coli*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed as described in BS EN ISO 20645 above.	has limited use as a simulation of final use of a treated material.
SN195924	Textile Fabrics - Determination of the Antibacterial Activity: Colony Plate Count Method	Fifteen replicate samples (each replicate is comprised of sufficient specimens of 25 ± 5 mm to absorb 1 ml of test inoculum) are inoculated with cells of either *E. coli* or *S. aureus* suspended in a liquid nutrient medium and incubated in sealed bottles for up to 24 hours at 27° C. After 0, 6 and 24 hours, 5 replicate samples are analysed for the size of the viable population present. A neutraliser is employed. An increase of 2 orders of magnitude of the population exposed to a control sample is required to validate the test. The method defines a textile as antibacterial if no more than a specified minimum level of growth is observed after 24 hours in 4 of the 5 replicate groups of samples.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN195921	Textile Fabrics - Determination of Antimycotic Activity: Agar Diffusion Plate Test	Replicate (4) samples of sterilised fabric (25 ± 5 mm diameter) are placed in intimate contact with a solid nutrient medium in a petri dish. Each petri dish has been prepared as a double layer. The first layer consists of 10 ml nutrient agar, the second layer of another 10 ml of the same nutrient agar to which 0.1 ml spore suspension (10^7 ml^{-1}) of either *Candida albicans*, *Aspergillus niger*, *Cladosporium sphaerospermum* or *Trichophyton mentagrophytes* had been added. The plates are then incubated at 28° C either 2 days (*C. albicans*) or 7 days (*A. niger*, *C. sphaerospermum* and *T. mentagrophytes*). The test is valid when control specimens of the same material without biocide, or of a biocide-free standard specified cotton material are fully overgrown. Good antimycotic efficacy is considered to be demonstrated when the specimens show no fungal growth on their	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		surface. The test specifies that both sides of a material have to be tested.	
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Absorption method	Replicate (6) samples of textile are inoculated with a standardised broth culture of either *S. aureus* or *K. pneumoniae* in individual tubes and then incubated at 37° C for 18 - 24 hours in closed containers. Samples are analysed for the presence of viable bacteria both before and after incubation by either total viable count or the determination of total ATP. Samples are sterilised prior to testing and a neutraliser is employed during recovery. The test is validated by growth of ^1 order of magnitude during the incubation period.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Transfer method	Replicate (6) samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *S. aureus* and *K. pneumoniae* using a 200 g weight for 1 minute. The samples are then removed. Replicate (3) samples are analysed for either the number of viable bacteria or the total ATM content both before and after incubation under humid conditions at 37° C for 24 hours. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either growth of ^1 order of magnitude during the incubation period or by a measure of the variability of the data obtained.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Printing method	Replicate (6) samples of test material are either *S. aureus* and *K. pneumoniae* by 'printing' cells collected on a membrane filter onto their surface in a standardised manner. The samples are then incubated under humid conditions for 18 - 24 hours at 20° C for a specified contact time(s). Replicate (3) samples are analysed for either the number of viable bacteria or the total ATM content both before and after incubation. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by	'Dry' inoculum intimate contact test. The transfer method of inoculation could be adapted to provide some simulation data.

		either determining the survival of the inoculum on the control material.	
ISO/FDIS 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 1 - Luminescence Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the ATP concentration associated with the samples. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced concentrations of ATP associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.
ISO/WD 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 2 - Plate Count Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the number of colony forming units. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced numbers of colony forming units associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.

Table VII: Methods used to Examine the Antimicrobial Activity of Carpets

Reference	Title	Description	Major Principle
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Qualitative Antibacterial Activity	Petri dishes with nutrient media are inoculated with a single, diagonal streak (approx.7.5 cm) of either *S. aureus* or *K. pneumoniae*. An unsterilized test specimen (25 mm x 50 mm) is placed in intimate contact and transversely across the inoculum on the agar surface. The plates are then inoculated at 37° C for 18 - 24 hours. The front and back of the carpet are tested separately. After incubation, the plates are inspected for the presence of growth both below the specimens and for any zone of inhibition surrounding the specimens. The test can also be used to test the effect of cleaning regimes. An untreated control is optional.	Qualitative assessment of rate of kill and zone diffusion test Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antibacterial Activity	Unsterilized specimens of carpet are pre-wetted with either sterile water or a wetting agent before being inoculated with individual suspensions of either *S. aureus* or *K. pneumoniae* in either a low or a high nutrient solution. The samples are then incubated in a tightly closed jar at 37° C for a specified contact time. Cells are recovered in 100 ml of a neutraliser after 0 and 6 - 24 hours of incubation. Activity is assessed by comparing the size of the initial population in the control (if used) with that present following incubation. A control is optional. When not employed, viable counts following incubation of the treated specimens alone are considered. The test can also be used to test the effect of cleaning regimes.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antifungal Activity	Petri dishes containing Sabouraud Dextrose Agar are inoculated with 1 ml of a spore suspension of *Aspergillus niger*. Immediately afterwards, specimens (38 mm diameter) of unsterile test material are placed into intimate contact with the agar. An additional 0.2 ml of the same spore suspension is also employed to inoculate the test pieces directly. The samples are	Zone diffusion test/surface growth test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

		then incubated at 28°C for 7 days. The back and front of the discs of carpet are tested in separate dishes. The zone of inhibition and the growth of fungus on the upper surface of the specimens are reported (no growth, microscopic growth, macroscopic growth). The test can also be used to test the effect of cleaning regimes.	
WIRA Test F	Test Method for Assessing the Survival of Test Organisms on Floor Coverings	Specimens (850 mm x 350 mm) are conditioned at 20°C and 65% RH before being subjected to 2 wet and 2 dry passes using a commercial spray extraction machine or a test rig. After 24 h drying, 12 specimens (each 60 mm diameter) are cut from the carpet. An aliquot (1 ml) of a suspension of cells of *E. coli* in nutrient broth is poured onto filter paper (7 cm diameter). The filter paper is then pressed for 1 min onto the surface of the carpet using a 1 kg weight. The filter paper is then discarded. After 0, 6 and 24 hours incubation at a specified temperature the carpet´s surface is pressed onto contact plates of McConkey agar. After 24h replicate (3) plugs (10 mm) are taken from each specimen and suspended in 10 ml nutrient broth for 30 seconds and then analysed for the presence of *E. coli* by total viable count.	Cell suspension intimate contact test. Potential to demonstrate the effectiveness of an antimicrobial treatment if appropriate incubation conditions are selected and addition species employed.

Table VIII: Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
JIS Z 2801: 2000	Antimicrobial products - Test for antibacterial activity and efficacy	The surface of replicate sample (3 for each treatment and 6 for the blank reference material - usually 50 mm x 50 mm) are inoculated with a suspension of either *E. coli* or *S. aureus* in a highly diluted nutrient broth. The cell suspension is then held in intimate contact with the surface by the use of a sterile polyethylene film (usually 40 mm x 40 mm) for 24 hours at 35° C under humid conditions. The size of the population on the treated surface is then compared with the size on the control surface both prior to and after incubation. A neutraliser for certain biocide types is employed. Antibacterial activity is certified if the difference between the lg_{10} of the population on the treated sample and that on the control surface is > 2.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 22196:2011	Plastics - Measurement of antibacterial activity on plastics surfaces.	This is the current New Work Proposal at ISO created from JIS Z 2801 by the SIAA of Japan. Modification and validation is in progress in collaboration with the IBRG. Some changes are expected.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité antibactérienne	Four replicate samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either *S. aureus* and *K. pneumoniae* using a 200g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37°C for 24 hours. A neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ASTM E2180-07	Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in	Replicate (3) samples of material are inoculated with cells of either *S. aureus* or *K. pneumoniae* suspended in molten semi-solid isotonic saline/agar. This attempts for form an 'artificial biofilm' which holds the suspension in intimate	Immobilised cell suspension intimate contact test. Basic efficacy test that

	Polymeric or Hydrophobic Materials	contact with the test surface of inherently hydrophobic materials. Samples are then incubated at a temperature similar to that intended for the final use for a specified period (usually 24 hours) under humid conditions. The size of the viable bacterial populations on the control and treated surfaces is then determined using a dilution plate count. Any effect is recorded using percent reduction calculated from the geometric means of the data. A neutraliser may be employed and sonication is used to separate the 'biofilm' from the test surfaces and suspend the agar gel. Subsequent imprinting of the test surface onto solid nutrient media can be performed to look for the presence of adherent viable cells.	has limited use as a simulation of final use of a treated material.
ASTM E2149-10	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of *Klebsiella pneumoniae* and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	Relies on either diffusion of antimicrobial agents from treated material into the cell suspension or due to interaction between the population and the surface of the material in suspension. Basic efficacy test that has limited use as a simulation of final use of a treated material.

Appendix 11. Information on the principle target organisms for PT 8 as outlined in the document (5.5.8)

Fungi

Wood rotting fungi

White rot/ brown rot fungi (*Basidiomycetes):*

Fungi responsible for brown rot (e.g. *Serpula lacrymans, Coniophora puteana*) and white rot (e.g. *Coriolus versicolor, Donkioporia expansa*)

Soft rot fungi (mainly *Ascomycetes, Deuteromycetes*)*:*

Fungi responsible for a type of rot characterised by surface softening of the wood although they also cause rot at depth *(e.g. Chaetomium globosum).* They are specifically significant for wood in ground contact.

Wood discolouring fungi

Sapstain*:*

The blue-black and brown discolouration of freshly felled logs or sawn timber have an economic importance. Sapstain causing fungi can only colonise wood as long as the sap wood contains enough water to provide solved sugars as a nutrient for these fungi ("green" wood). Therefore, these fungi can be controlled by rapid drying of the wood after felling, chemical treatments are sometimes used.

Common sapstain species include e.g. *Stereum* spp*.,* blue staining species.

Blue stain cause blue to black permanent colour of variable intensity and depth mainly in the sapwood depending on the wood species. This does not result in appreciable alteration of the mechanical properties but can increase the permeability of the wood and thereby makes it more susceptible to fungal degradation.

Common blue staining species include e.g. *Aureobasidium* spp*., Ceratocystis* spp.

Mould fungi:

Fungi, e.g. *Aspergilus* spp*.*, *Penicillium* spp*.* being evident as spots of various colours on the surface of moist wood. (for instance, as a result of high relative humidity or of condensation of water vapour). They do not significantly alter the mechanical properties of the wood but have a special significance for wood in service if discoloration is undesirable or unacceptable.

For green sawn timber, the moulds are covered by the CEN TS 15082 standard. But for the preservation of solid wood against mould, the EN 152 does not cover mould and no CEN standard is available. In that case the applicant is invited to submit relevant data (in house method, literature data...) which could be accepted by expert judgement.

Insects

Fresh wood insects

A number of insects bore and tunnel into fresh logs after they are cut and debarked. These fresh wood insects feed upon the starch reserves and can cause damages to the wood. Most of them belong to the families of Scolytidae (genus *Scolytus*), Cerambycidae (genus *Phematodes*), Lyctidae (genus *Lyctus*), Anobiidae (genus *Anobium*), Bostrychidae (genus *Bostrychus*).

Some other groups, belonging to the Scolytidae family, bore the fresh logs and introduce 'Ambrosia' fungi inside the gallery, resulting in wood staining (as a consequence of the development of the dark hyphae).

Wood boring beetles (Coleoptera)

Insects which lay their eggs in wood pores or cracks and whose larvae feed upon wood. They are present throughout Europe but the risk of attack varies greatly and is ranged from high to insignificant. The most important are *Hylotrupes bajulus*, *Anobium punctatum* and *Lyctus brunneus*.

Hylotrupes bajulus (House longhorn beetle)

This beetle attacks many softwood species and can cause significant structural damage. Many softwood species are affected, whereas hardwoods are not attacked. Larvae damage both the sapwood and the heartwood of non durable species.

This insect occurs throughout Europe, but is of less importance in the north and north-west of Europe. The vitality and longevity of larvae depend principally on ambient temperature and the wood moisture content.

Anobium punctatum (Common furniture beetle)

The larvae attack the sapwood of certain softwood and hardwood species. The damage can extend to the heartwood in some wood species and can have occasionally a structural significance impact. Its presence is particularly noted in coastal climates and where damp conditions prevail.

Lyctus brunneus (Powder post beetle)

The larvae attack sapwood of certain starch-containing hardwoods and have a significant impact throughout Europe for both European and imported hardwood timbers.

Termites (Isoptera)

Termites belong to the order Isoptera. In Europe and in the European tropical overseas regions there are three main termite families; subterranean termites (Rhinotermitidae), drywood termites (Kalotermitidae) and tree termites (Nasutitermitidae):

- *Reticulitermes* is the most common genus encountered from the Rhinotermitidae family in Europe. The main species registered are: *R. flavipes* (former *R. santonensis*), *R. grassei, R. lucifugus*, *R. banyulensis, R. balkanensis, R. urbis.*

  They are widespread around the Mediterranean basin (Spain, France, Italy, Portugal, Balkans, and Greece) and Black Sea (Turkey, Romania), though some termite spots in the UK or Germany have been reported. Several unanswered questions remain about the origin of these termites. While some *Reticulitermes* are native to Europe, others may be related to species from eastern North America and the Middle East (Israel, Asian Turkey, etc.).

  Coptotermes and *Heterotermes* are the main two genera belonging also to the Rhinotermitidae family located in the European tropical overseas regions.

- *Kalotermes flavicollis* and *Cryptotermes brevis* are the main two species of drywood termites present in Europe (especially in the coastal areas of Mediterranean countries and Canary Islands). *Cryptotermes* is a main genus belonging to drywood termites encountered in the European tropical overseas regions.

- *Nasutitermes* is the main genus belonging to the Termitidae family (tree termites) encountered in the European tropical overseas regions.

Marine borers

This term is applied to marine invertebrates such as *Limnoria* spp. *and Teredo* spp. which need a certain salinity of water and which hollow out extensive tunnels and cavities in wood. These organisms can cause serious damage to fixed or floating structures.

In European waters the most common marine borers are shipworm (*Teredo navalis*) and gribble (*Limnoria* spp.). Shipworm is a bivalve mollusc related to the sea snails and mussels. It is a soft, worm like animal with its shell modified into hard grinding jaws. The larvae are part of the microscopic zooplankton and swim freely in the sea until they settle on timber. They develop a shell with which they bore into the wood and lodge there, growing into large worms in holes up to 5 mm in diameter. They destroy the wood by making a massive network of galleries throughout the timber. Gribble is a small shrimp-like crustacean about 4 mm in length. It bores into the surface of the wood and lodges near the surface making numerous side burrows. The combination of this boring and wave action causes rapid erosion of marine timbers.

Appendix 12. Annex A of EN 599-1

Introduction

Additional explanations regarding Annex A of the standard EN599-1:2009+A1 :2013, mainly on cases where no new biological testing is necessary, are presented here following the sections of the Annex.

NOTES to the reader:

Annex A is not a normative Annex in EN 599-1 but only informative and intended to act as guidance.

Annex A of EN 14128: the majority of the points (below) also apply to Annex A of EN 14128, except for Section A.3; additional notes will be added at a future update of this guidance document.

In this Appendix, a "ready-to-use formulation" refers to the product as marketed; this includes concentrated products (which are diluted before application) and products which do not need to be diluted before application, (i.e. they can be used directly from the container). The efficacy will be demonstrated at the concentration used.

The introduction to Annex A lists the modifications which can occur during the development of a product for the first or subsequent authorisations (e.g. minor change, major change).

The composition of the products as tested is the basis to assess the variations that can occur in a biocidal product family (BPF).

Variations can occur within a BPF which fall outside some of the guidelines given in Annex A and which may require additional efficacy testing even if the products are considered within the same BPF; nor should Annex A be considered as being only applicable to a BPF.

This Appendix has been written to give guidance on whether existing test results could still be considered valid where formulation changes have been made or when additional laboratory testing according to the provisions of EN 599 may be required. This is a helpful and pragmatic approach regardless of whether a BPF is being considered or a first or subsequent authorisation.

Sections of Annex A– additional explanation

Paragraph A.2 No requirements for new biological testing

Section A.2.1

This section lists all the allowed variations from the tested formulation of the BP (given in sub-sections A.2.2, A.2.3 and A.2.4), for which no new biological testing is required. It should be clarified if only one variation is allowed between the two products or if several variations are allowed.

→ all of the variations may occur (and are allowed).

Section A.2.2 In the case of organic solvent based products (ready for use)

- **Sub-section A.2.2 a**

  Changes involving substitution of any co-formulant by one which is chemically equivalent from another supplier.

→ "Substitution" means replacement of a chemically equivalent co-formulant performing the same function in the product formulation.

"Chemically equivalent" means that chemicals have the same CAS number and the same physical properties (e.g. pH, molecular weight distribution (for polymers), HNL number (for surfactants). It is **a chemical from another supplier**.

Information on function of the co-formulant should be provided; co-formulants are any ingredient other than an active ingredient, in a formulated wood preservative product. Typical chemical functions for non-active ingredients of wood preservative can be, for example, solvents, surfactant, emulsifier, corrosion inhibitor, binder, pH stabiliser, mordant, dye, pigment, 'penetration marker' water repellent and co-solvent.

- **Sub-section A.2.2 b**

  Products to be applied by penetrating treatment processes for changes in the aromatic content or chemical nature of hydrocarbon solvent carriers[65], providing that not less than 90% (v/v) of the carrier distils below 250°C.

  → The reason for such a change is that a product could have been tested (e.g. in an EN113 test) using the organic solvent xylene. The xylene evaporates during drying of the treated blocks leaving the active substance in the dry wood blocks, so the solvent does not affect the efficacy of the product. The blocks are exposed to the test fungus and the Biological Reference Value (BRV) and the Critical Value (CV) for the product are determined. This point in Annex A allows an organic solvent based product containing the active substance to be formulated (using an aromatic substance such as 'Caromax 18', 'white spirit', 'Stoddard solvent' or 'odourless kerosene' to dissolve the active substance) without retesting the product. The principle is that the organic solvent evaporates after treatment, leaving the product solid at, or above, the CV and the type and composition of the organic solvent carrier does not affect the efficacy of the product. Thus an efficacy test of a product (e.g. EN 113, EN 47) with a xylene solvent/carrier can be used to confirm the efficacy of an organic solvent based product applied by a penetrating process with a different solvent carrier, providing that not less than 90% (v/v) of the carrier distils below 250°C. If the data are not available (e.g. the SDS of an hydrocarbon solvent carrier does not contain information about boiling point), a justification should be provided by the applicant.

- **Sub-section A.2.2 c**

  Product to be applied by superficial processes, for a change in the aromatic content of hydrocarbon solvent carriers of no greater than 10% (v/v of the total aromatic hydrocarbon solvent content).

  → See point A.2.2 point b.

  Example:

  A formulation tested (e.g. EN113) with 20% m/m aromatic hydrocarbon solvent/carrier, can be read-across to a biocidal product containing no less than 18% and no more than 22% total aromatic hydrocarbon solvent (= ± 10% of 20%).

[65] The carrier is the substance used to convey the wood preservative formulation into the wood e.g. water or organic solvent (see for example EN 599-1 A2.2(b)). A solvent may be used as the main carrier but may also be used for other purposes within the wood preservative formulation (e.g. as part of a micro-emulsion). In the latter case, EN 599-1 refers to this as a "co-solvent" (see for example EN 599-1 A2.3(f)).

- **Sub-section A.2.2 d + e**
  - **Changes involving the addition or deletion of a soluble dyestuff.**
  - **Changes in pigments to an equal or lower pigment content of the product.**

  → "Soluble dyestuffs" ('dyes' in the BPR) are coloured, non-biocidal **soluble** substances which do not impede the flow of liquid through the wood structure; this is so that they do not reduce penetration of the active substances in a wood preservative and do not affect the efficacy of an active substance or biocidal product. Dyes may be included in a wood preservative as a penetration marker to differentiate between treated and untreated timber and/or to colour the preserved wood.

  "Pigments" are coloured, non-biocidal, **insoluble** materials, dispersed in a suitable medium. Some pigments have been found to reduce the penetration of the active substances in a wood preservative.

  Due to the **potential** impact of pigments on penetration it was decided to allow changes only up to the former content of pigment (solid portion) in the formulation when the 'no additional testing rule' must apply. If the exact content of the pigment and its solid portion is unknown changes up to the total content of the pigment paste are allowed if robust justification is provided.

  It can be accepted to test a formulation without pigment.

  In cases where additional pigments are used in the product, it has to be demonstrated that the conditions of A.2.5 are fulfilled.

- **Sub-Section A.2.2 f**

  Product containing 10% (m/m) or less of solids containing resins and/or water repellents[66], relative changes in content of these constituent(s) of no more than ± 20% (m/m) and products containing more than 10% (m/m) solids, relative changes of no more than ± 10% (m/m).

  → With reference to wood preservative formulations, a solid is the proportion of non-volatile material contained in a formulation after the volatile solvent, (which serves as a carrier or vehicle for the solid content) has vaporized or evaporated.

  A "resin" is a non-volatile organic polymer and can be solid, semi-solid or liquid form.

  An ingredient can be considered to make up the 'solid' portion of the preservative if it is non-volatile. However, in this section the solid content being referred to are specifically resins plus water repellents. Solids of pigments are excluded here and dealt with in A.2.2e.

  Example of a calculation of the allowed variations in case of a product containing resin and water repellent:

  > For a product containing 5% resin + 7% water repellent (non-volatile portion) then the allowed variation is: (5+7) * 10 / 100 = ± 1.2%.

[66] Water repellents are co-formulants in a formulation impart additional resistance to the absorption of water by the treated wood product. Typically water repellents are, but not limited to, of waxes or silicon base.

- **Sub-Section A.2.2 g**

  Up to 5% of the hydrocarbon solvent may be replaced by a solvent miscible co-solvent fulfilling the distillation range given in sub-Section A.2.2 b

  If the content of pigments in a formulation is not available (e.g. not provided in the SDS), the content of the pigment mixture should be applied. For products that cannot benefit from this approach, additional data on the solid pigment content should be requested from the applicant.

- **Sub-Section A.2.2 h**

  Adding and/or replacing a co-formulant providing the additive constitutes less than 2% of the total formulation and providing the physical properties are not affected (A.2.5).

  →"Replacing" means changing one co-formulant for another. Partial replacement is permissible.

  "Adding" refers to both the addition of a new co-formulant and to the increase of an existing co-formulant.

  The 2% relates to each individual substance. This value was chosen on the basis that it represents a safe level of change within a formulation that experts were confident would not affect the efficacy of a formulation, provided that stability was unaffected (hence the requirement that the provisions in A.2.5 should be met).

  An example of formulation modification to illustrate this section could be the exchanged/amended amount of propylene glycol with ethylene glycol by a change of ± 2%.

Section A.2.3 In the case of water-soluble preservatives

- **Sub-section A.2.3 a:** see A.2.2 a
- **Sub-section A.2.3 b:** see A.2.2 d
- **Sub-section A.2.3 c**

  For products in their <u>ready for use</u> form containing 10% (m/m) or less of solids containing resins and/or water repellents, relative changes in content of these constituent(s) of no more than ± 20% (m/m) and for products containing more than 10% (m/m) solids, relative changes of no more than ± 10% (m/m)of these constituents.

  See also Sub-section A.2.2 f for the definitions of "solid" and "resin".

- **Sub-section A.2.3 d**

  In a case of inorganic active substances (e.g. copper II salts), no additional biological testing is required when changing the inactive component (the anion part) of the active substance not resulting in a change in the ratio, total content or chemical properties of biocidal active component (e.g. copper II).

- **Sub-section A.2.3 e:** see A.2.2 e
- **Sub-section A.2.3 f**

  Changing or adding a water miscible co-solvent (distillation ranged as in A.2.2 b) up to 5% of the total formulation.

- **Sub-section A.2.3 g:** see A.2.2 h

Section A.2.4 In the case of emulsion products

→ Differentiation between water soluble preservative (2.3) and emulsion products (2.4) Often products are part suspension and part emulsion.

At the time of the development of EN 599, emulsion concentrates were a relatively new technology. This explains why all the comparisons were made in relation to water-borne preservatives. With the knowledge and widespread experiences nowadays this separation is not justified anymore. It is recommended that section A2.3 is used for all water-based preservative formulation types (i.e. solution/emulsion/suspension or combinations of these) while ensuring the physical form of the active substance in the formulation is unchanged (i.e. solution/emulsion/suspension).

See section A.2.3.

Section A.2.5.

- **For the sub-sections A.2.2 h, A.2.3 g, , it should be confirmed that:**
  - **the penetration into the wood is not adversely affected (only for penetrative treatments);**
  - **the stability of the product is not adversely affected; this can be demonstrated e.g. with the chemical analysis, of the active ingredients after storage stability.**

→ If, after a formulation change, an improvement in stability is recorded through chemical analysis after storage at 40 degrees C, this in itself will not result in a requirement for additional testing.

→ You cannot generally predict the penetration of a wood preservative product from its composition. The combination of product composition and application process governs the wood preservative penetration.

Laboratory scale or pilot plant trials using standard timber species and standard process cycles would be appropriate to demonstrate that the penetration into the wood is not adversely affected.

Accelerated storage stability tests can be used to fulfil the requirements for the stability of the product and active substance content after storage.

Paragraph A.3 Requirement for minimum new biological testing

Practical case: Is it possible to combine section A.3 and A.2?

Example:

- **Product A is a fungicidal and insecticidal product. Data on the efficacy of this product is available;**
- **Product B is insecticidal only and the composition is very close to the product A except the fungicide active substance deleted and one compound added to the formulation A (at 1.5% w/w).**

  When it is taken into account that efficacy data demonstrate that the fungicidal active substance has no impact on the insecticidal active substance and that the point A.2.3 and A.2.5 are fulfilled. Is the double read-across acceptable?

  - A2 and A3 are for different situations;

- A2 specifies conditions where there is no requirement for new biological testing;
- A3 specifies conditions for minimum new biological testing (though in the case of changes to fungicide and insecticide levels it also describes instances where no additional testing will be required).

In the example, the data provide sufficient demonstration of the effectiveness of Product B against insects.

The 'double read-across' is acceptable. The results from the insect efficacy studies for Product A can be read-across to Product B according to Annex A of EN599. This is acceptable because the addition of the compound to Product A is less than 2% w/w. Assuming that the description of the function of the compound in the 'Identity' section is acceptable under Annex A, because it will not adversely affect penetration, Product B does not require retesting under Annex A and Product B can be considered to be effective against insects under BPR.

Under Annex A, the fungicidal active substances could be omitted from Product B without retesting the efficacy of Product B against insects if data exist which confirm that the removal of the fungicide does not affect the insecticidal efficacy (section A.3.2.2). Product B (without fungicide) can only be claimed to be effective against insects, and the insect studies for Product A can be used to confirm the effectiveness of Product B against insects.

Appendix 13. Laboratory studies for rodenticides: bait choice test

This appendix describes a protocol of a laboratory study to determine the efficacy of an as yet unauthorised product (rodenticide) against the house mouse, brown rat and roof rat containing a bait formulation. This protocol can be applied to other target organisms (e.g. voles).

A feeding test is conducted to determine the extent to which rodents will eat the product when they are given a free choice between that and their normal food. This type of palatability test is most suited to slow-acting toxicants. The test consists of an acclimatisation period, followed by a pre-test diet take assessment, then a test period of normally[67] 3-5 days and at least 14 days of post-treatment observation.

Pre-test period

For the test, normally 10 wild or laboratory strain rodents (5 males and 5 females) are required. Laboratory rodents should be healthy, non-pregnant adults of known strain (STATE). Preferably wild adult rodents are used. They should be healthy and obtained from free-living populations (STATE WHERE) in accordance with Directive 2010/63/EU, Articles 7 and 9 and Section A, 3.2 of Annex III . On arrival at the laboratory, the wild strains should be treated with an appropriate insecticide to kill ectoparasites and then be housed in small groups (no more than five per cage) of the same sex and treatment group if no aggressive behaviour is expected, preferably in solid floor cages with appropriate environmental enrichment. Animals may be housed individually only if scientifically justified. With wild rats especially, it is advisable to place all items (i.e. food pots) required for the test in the cage before each animal is released into it. Wild rodents should be acclimatised to laboratory conditions for at least 3 weeks to ensure that no females are pregnant when the test begins. During this time they should be offered a laboratory animal diet and water should be freely available. To encourage variation in response, animals with body weights throughout the range normally expected for the species should be used as far as possible.

Before the test period begins, it is necessary to ensure that the animals are feeding normally. Following acclimatisation, two food pots, placed either side at the front of the cage, are filled with cereals, such as wheat, broken wheat, or a wheat-based mixture or ground laboratory diet or EPA meal. All other food is removed, but water remains freely available. The quantity of food placed in each pot (STATE) should be sufficient to meet each animal's daily needs. Food uptake should be determined, therefore all unused food (i.e. food left in the pot) and scattered food must be collected and taken into account by weighing to determine how much of the food has not been eaten. All unused diet (i.e. food left in the pot and scattered food) should be discarded and the pot refilled with a fresh supply, to ensure it is palatable. This procedure should be repeated for a further 3 days and on the last day (of this pre-treatment period) the animals should be weighed. Also on the last day, the diet remaining in each pot and scattered food, is weighed and the total amount of food eaten by each rodent calculated (STATE). Any rodent not eating normally by the last day should be discarded.

Test period

The palatability test commences with 2 clean bait containers, one filled with a quantity of the test product and the other with a suitable challenge diet (e.g. an EPPO challenge diet[68] or standard laboratory diet). Again, the quantity in each pot should exceed the

[67] Deviation from this norm is possible but should be explained in the application.

[68] EPPO guideline PP1/113 for the efficacy of rodenticides, Laboratory tests for evaluation of the toxicity and acceptability of rodenticides and rodenticides preparations. Revised 1998.

normal daily requirement for each animal. After 24 hours, the diet remaining in each pot is weighed and the total amount of food eaten by each rodent calculated. All used test and challenge diet is discarded and fresh quantities of each diet are placed in clean pots. In placing the pots back in the cage, the positions of the rodenticide and the challenge diet should be interchanged to avoid place preference. This procedure should be repeated every day during the choice period. After day 4 (3 or 5 is also acceptable) the animals should be returned to the standard laboratory diet.

Observation period

During the observation period the rodents are observed at least once per day and any signs of toxicity and mortality are recorded. Humane end-points should be applied in line with Directive 2010/63/EU to all animals showing clinical signs that can determine impending death.

Guidance Document on the recognition, assessment and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (OECD, 2002) must be considered.

Results

Results should be shown as the percentage intake of rodenticide and the percentage intake of challenge diet (see section 2.2.1 for further details). Also the percentage mortality and any other symptoms should be mentioned.

Liquid bait formulations

The test must be carried out as above with the following exceptions:

- a suitable compounded laboratory diet shall be freely available;
- tap water must be used as the control bait;
- all procedures relating to the solid control and test baits must be applied instead and as appropriate to the liquid control and test baits;
- when the positions of the test and control baits are interchanged the positions of the drinking tubes, if used, should not be interchanged;
- liquid baits must be provided in containers with non-drip nozzles or suitable open pots;
- a filled container must be placed out of reach of the animals in order to monitor weight loss due to evaporation.

Appendix 14. Field trial for rodenticide baits

This appendix describes a protocol and factors to be taken into account when conducting a field trial to determine the efficacy of an as yet unauthorised rodenticide bait product against the house mouse, brown rat or roof rat. This protocol can be applied to other target organisms (e.g. voles).

Ideally field trials should:

- be conducted with separate rat and mice populations (as appropriate to the intended uses in the draft SPC);
- be carried out at sites that are representative of the intended uses in the draft SPC (for example industrial, commercial, domestic);
- include sites with 'known' anticoagulant resistant populations (if appropriate to the intended uses in the draft SPC);
- have had no rodenticide treatments over the past 6 weeks;
- Incorporate lag phases before and after the treatment phase;
- for testing concentrates, cover a range of bait bases;
- for product that is sold with a specific bait station, include the whole device (the bait and its station) in the test;
- be carried out at 2 or 3 locations (i.e. a trial site sufficiently far away from the next, dependent on the roaming pattern of the test organism; e.g. Sites >30 m apart for Norway rats (Buckle and Smith 2015).

The following suggested method for bait formulations details the extent of the data required, but the methods may be replaced or supplemented by new techniques as appropriate.

Suggested procedure for bait formulations

Trial sites

Each trial site should, as far as possible, comprise a discrete infestation of one target species, with little chance of rapid reinvasion from adjoining areas.

During the entire trial, the baiting sites should be at exactly the same locations, taking into account distances as specified in the intended use, local structure and rodent activity as established prior to the trial. See also the Good Practice Document released by Cefic (http://www.cefic.org/Documents/Industry%20sectors/EBPF/Guideline-on-Best-Practice-in-the-Use-of-Rodenticides-in-the-EU.pdf), and the field trial protocol released by the RRAC (www.rrac.info/releases/technical-monographs/).

At each baiting site, a bait container is placed, the top of which is closed/covered, to protect the bait from weather and avoid spillage. When selecting baiting sites, it is important that the animals can feed without being disturbed.

The amount of bait applied in each feeding point should correspond to the amount given in the use instructions in the draft SPC. In general, for mice, the amount of bait applied in each feeding point is less than for brown or roof rats. In other respects, the test design is identical for both groups. It is important that there is always enough fresh food or bait containing the active substance present.

Before the trial begins, draw a sketch map showing all significant features of the site including signs of infestation.

Data on field efficacy is likely to be more reliable if infestations of brown rats and house mice are selected on the basis that a stable level of activity is obtained during the pre-treatment assessment. The level of activity can be determined by two of the following (as appropriate to the situation, species etc.):

- census baiting;
- tracking techniques;
- census by live trapping;
- electronic methods of census.

Pre-treatment activity measurement/estimation of numbers

Indices of the target species population should be obtained both before and after the test treatment normally by at least 2 of the following quantitative methods. Other methods, such as electronic remote detection systems, can be used as additional information for example, in combination with bait census.

Pre-treatment bait census
The position of the census bait points should be indicated on the site sketch plan. Census bait should be laid for at least 4 days to cover the whole infestation in quantities at each bait point which as far as possible exceed the maximum daily take by rodents. The number of census baits should be approximately the same as the planned number of test bait points. Census points should not be located at the same place chosen to lay poison points but should be at different (intermediate) positions. Census bait should be different to the bait base used in the test product.

The number of points where take has occurred and the amount of the take of the census bait, should be recorded daily. An indication of the change in weight of the bait due to moisture loss or uptake should be included.

At the end of the bait census all baits and containers should be removed from the trial site. The total amount of census bait consumed will give an index of population size.

Tracking activity measurement
This is recommended for both rats and mice, and should be measured over at least 3 days, simultaneously with the bait census, using tracking patches/boards laid around the site in numbers similar to the census bait points but as far as possible, not in the same locations. The locations of the patches/boards should be indicated on the plan.

The patches/boards should be inspected for signs of activity and resurfaced daily. A simple scoring system can be devised to assess the number of rodent footprints per patch/board: summing the individual scores gives a daily activity index. When the pre-treatment assessment is complete, the tracking patches/boards may be removed from the site or maintained to provide supplementary information on rodent activity.

Census by trapping
This is recommended for mice only, and should be carried out for a period of at least 3 days using rodenticide-free bait in the live traps. Live traps should be laid around the site in numbers appropriate to the situation and likely population size.

Animals caught should be marked by fur clipping and subsequently released. The numbers caught should be recorded and used to estimate the size of the population.

The live traps should then be removed from the test site during the rodenticide treatment.

Lag period

Once the pre-treatment population measurement has been conducted there should be a lag period, normally 3-14 days (or longer for acute poisons where no pre-baiting is recommended) with no experimental interference (other than tracking) on the site.

Test treatment

The test formulation must be applied in accordance with the draft SPC for an appropriate period (normally[69] 4 days for acute products and 30-40 days for multi-dose products). The locations of test bait points should, as far as possible, be different from those of the census bait points, traps, and tracking patches/boards.

Where applicable the following items should be recorded:

- the locations of the bait points on the plan;
- the amount of bait deposited at each point at each visit and the amount retrieved, including details of the type of container used;
- the number and species of rodents and other animals found dead, and the dates on which they were found;
- the dates of all observations, treatments and censuses;
- any other information deemed relevant. This may include, for example weather conditions, temperature data, site changes instituted by the occupier (including improvements in hygiene and proofing), or supplementary information on rodent tracking activity.

On termination of the treatment all poisoned baits and bait containers should be removed from the trial sites. Similarly rodent bodies should be searched for, removed and disposed of in the appropriate way for example, burial or burning.

Post-treatment lag period

On completion of the treatment there should be a lag period sufficient to allow poisoned animals to die or survivors to recover from the sub-lethal effects of the rodenticide. This period may be 3-14 days, depending on previous observations of time to death or full recovery. During this period there should be no experimental interference with the site other than tracking.

Post-treatment activity measurement/estimation of numbers

Once the post-treatment lag period is completed, the methods employed to measure pre-treatment activity should be conducted in exactly the same way. Traps, baits and tracking patches should be laid in exactly the same places as in the pre-treatment census.

After each field trial, a comparison of population indices before and after treatment determines how successful the product has been in controlling the target population. The degree of control is expressed as a percentage reduction in the pre-treatment index.

[69] Deviation from this norm is possible but should be explained in the application.

Appendix 15. List of currently available standard test methods for rodenticides

This list may not be exhaustive, and makes no comment on the suitability of particular test methods for efficacy testing.

Table 47: List of standards

Standard	Title	Target Organism(s)	Mode of Application
EPA/OPP Protocol Number 1.201	Standard Norway Rat and Roof Rat Anticoagulant Liquid Bait Laboratory Test Method	Brown Rat/Roof Rat	Liquid bait
EPA/OPP Protocol Number 1.202	Standard House Mouse Anticoagulant Liquid Bait Laboratory Test Method	House Mouse	Liquid bait
EPA/OPP Protocol Number 1.203	Standard Norway Rat and Roof Rat Anticoagulant Dry Bait Laboratory Test Method	Brown Rat/Roof Rat	Dry Bait
EPA/OPP Protocol Number 1.204	Standard House Mouse Anticoagulant Dry Bait Laboratory Test Method	House Mouse	Dry Bait
EPA/OPP Protocol Number 1.205	Standard Norway Rat/Roof Rat Anticoagulant Tracking Powder Efficacy Laboratory Test Method	Brown Rat/Roof Rat	Tracking Powder
EPA/OPP Protocol Number 1.212	Standard House Mouse Anticoagulant Tracking Powder Efficacy Laboratory Test Method	House Mouse	Tracking Powder
EPA/OPP Protocol Number 1.213	Standard Norway Rat/Roof Rat Anticoagulant Wax Block and Wax Pellet Laboratory Test Method	Brown Rat/Roof Rat	Wax Block and Wax Pellet
EPA/OPP Protocol Number 1.214	Standard House Mouse Anticoagulant Wax Block and Wax Pellet Laboratory Test Method	House Mouse	Wax Block and Wax Pellet
EPA/OPP Protocol Number 1.217	Standard Norway Rat and Rood Rat Anticoagulant Placepack Laboratory Test Method	Brown Rat/Roof Rat	Placepack dry bait
EPA/OPP Protocol Number 1.218	Standard House Mouse Anticoagulant Placepack Penetration Laboratory Test Method	House Mouse	Placepack penetration
EPA/OPP Protocol Number 1.221	Proposed Norway Rat Anticoagulant Technical and Concentrated Dry Bait Laboratory Test Method	Brown Rat	Technical and Concentrated Dry Bait
EPA/OPP Protocol Number 1.225	Proposed House Mouse Anticoagulant Technical and Concentrated Dry Bait Laboratory Test Method	House Mouse	Technical and Concentrated Dry Bait
EPA/OPP Protocol Number: 1.207	Standard Norway Rat/Roof Rat Acute Liquid Bait Laboratory test method	Brown Rat/Roof Rat	Liquid bait
EPA/OPP Protocol Number: 1.208	Standard House Mouse Acute Liquid Bait Laboratory Method	House Mouse	Liquid bait
EPA/OPP Protocol Number: 1.209	Standard Norway Rat/Roof Rat Acute Dry Bait Laboratory Test Method	Brown Rat/Roof Rat	Dry Bait
EPA/OPP Protocol Number: 1.210	Standard House Mouse Acute Dry Bait Laboratory Test Method	House Mouse	Dry Bait
EPA/OPP Protocol Number: 1.211	Standard Norway Rat/Roof Rat Acute Tracking Powder Efficacy Laboratory Test Method	Brown Rat/Roof Rat	Tracking Powder

Standard	Title	Target Organism(s)	Mode of Application
EPA/OPP Protocol Number: 1.219	Standard Norway rat/Roof rat Acute Placepack Penetration Laboratory Test Method	Brown Rat/Roof Rat	Placepack penetration
EPA/OPP Protocol Number: 1.220	Standard House Mouse Acute Placepack Dry Bait Laboratory Test Method	House Mouse	Placepack dry bait
EPA/OPP Protocol Number: 1.222	Proposed Norway Rat Acute Technical and Concentrated Dry Bait Laboratory Test Method	Norway rat	Technical and Concentrated Dry Bait
EPA/OPP Protocol Number: 1.226	Proposed House Mouse Acute Technical and Concentrated Dry Bait Laboratory Method	House Mouse	Technical and Concentrated Dry Bait
EPA/OPP Protocol Number: 1.227	Proposed House Mouse Acute tracking Powder Efficacy Laboratory Method	House Mouse	Tracking Powder
BBA 9 - 3.1	Richtlinie für die Prufüng Prüfung von Nagetierbekämpfungsmitteln gegen Hausmause	House Mouse	Dry and liquid bait, wax block and pellets, contact rodenticides
BBA 9- 3.2	Richtlinie für die Prüfung von Nagetierbekämpfungsmitteln gegen Wanderratten	Brown Rat	Dry and liquid bait, wax block and pellets, contact rodenticides
EPPO 1982	Guidelines for the Biological Evaluation of Rodenticides No1. Laboratory Tests for Evaluation of the Toxicity and Acceptability of Rodenticides and Rodenticide Preparations	-	-
EPPO 1982	Guidelines For the Biological Evaluation of Rodenticides. Field Tests Against Synanthropic Rodents (*Mus musculus, Rattus norvegicus, Rattus rattus*)	-	-
EPPO 1986	Guidelines for the Biological Evaluation of Rodenticides. Laboratory and Field Tests for the Evaluation of Rodenticidal Dusts	-	-
ASTM E 565-95	Standard Test Method for Efficacy of a Single-Dose Acute Rodenticide Under Laboratory Conditions for Commensal Rodents	Brown rat/Roof rat/ House mouse	Dry Bait
ASTM E 593-95	Standard Test Method for Efficacy of a Single-Dose Acute Rodenticide Under Laboratory Conditions	Brown rat/Roof rat/ House mouse	Dry Bait
EPPO Standards/97(2)	Laboratory and field tests for the evaluation of rodenticidal dusts	-	-
EPPO Standards /113(2)	Laboratory tests for evaluation of the toxicity and acceptability of rodenticides and rodenticide preparations	-	-
EPPO Standards /114(2)	Field tests against synanthropic rodents	Brown rat/Roof rat/ House mouse	-

Standard	Title	Target Organism(s)	Mode of Application
EPPO Standards /169(2)	Efficacy trials with rodenticide baits under practical conditions against Voles (*Arvicola terestris and Microtus* spp.) in their subterraean galleries"	Voles (Microtus, Arvicola)	-
EPPO Standards /197(1)	Non-target effects of rodenticides	-	-
EPPO Standards /198(1)	Testing rodents for resistance to anticoagulant rodenticides	-	-
RRAC rat field trial protocol 2013	Field Trial to Evaluate the Efficacy of Rodenticide Baits for the Control of Rats (*Rattus norvegicus*)	Brown Rat/Roof Rat	Dry Bait
OECD	OECD Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Human Endpoints for Experimental Animals Used in Safety Evaluation (2002) http://www.oecd-ilibrary.org/environment/guidance-document-on-the-recognition-assessment-and-use-of-clinical-signs-as-human-endpoints-for-experimental-animals-used-in-safety-evaluation_9789264078376-en	-	-
EPPO Standards PP1 2004	2nd edition, volume 5, EPPO, Paris (2004), 48-56.	Voles	-
BBA (1963)	Richtlinie 9-2, Richtlinien für die Prüfung von Nagetierbekämpfungsmitteln gegen Schermaus (in German)	Voles	-
BBA (1980)	Richtlinien für die amtliche Prüfung von Pflanzenbehandlungsmitteln 18-3.3, Richtlinie für die Prüfung von Rodentiziden gegen Schermaus im Forst (in German)	Voles	-
Méthode CEB n°254 (2013)	Méthode d'essai d'efficacité pratique de générateurs de gaz fumigants pour lutter contre la taupe (*Talpa europaea*) et le campagnol terrestre (*Arvicola terrestris*) dans leurs galeries souterraines au champ.	Voles, moles	Gassing agent
Méthode CEB n°257 (2014)	Méthode d'essai d'efficacité pratique d'appâts rodenticides pour lutter contre les campagnols (A*rvicola terrestris, Microtus* spp.) dans leurs galeries souterraines au champ	Voles, moles	Bait

Appendix 16. Additional information on label claims

NOTE to the reader:

This Appendix contains some information referring to the Biocidal Product Directive that is now obsolete. This will be revised at the next update, but in the meantime, readers should use the information from this Appendix in conjunction with the information available in Section 2 on Claims and also in Section 5 in the general sections and under each PT section. The text in these sections has been revised and updated.

Assessing the efficacy of biocidal products

The evaluation of the efficacy of biocidal products differs greatly from that of active substances.

Whilst the efficacy assessment of an active substance for Annex I inclusion requires only a minimal assessment, sufficient to show an innate level of activity for the active substance, the assessment needed for a biocidal product at the product authorisation stage is much more detailed.

Rather than looking at innate effects, the efficacy assessment of a biocidal product is based on substantiating the efficacy claims made for a product. The assessment is made on the product in its normal conditions of use.

This principle is set out in paragraph 51 of Annex VI of the Directive (Common Principles for the Evaluation of Dossiers for Biocidal Products), which states:

> *5.1 Data shall be submitted and evaluated to ascertain if the efficacy claims of the biocidal product can be substantiated. Data submitted by the Applicant or held by the Member State must be able to demonstrate the efficacy of the biocidal product against the target organism when used normally in accordance with the conditions of authorisation.*

The label claims for the product must be submitted as part of the common core data set, as set out in Annex IIB (Common Core Data Set for Biocidal Products), which requires:

> *V. INTENDED USES AND EFFICACY*
>
> *5.10. The proposed label claims for the product and efficacy data to support these claims, including any available standard protocols used, laboratory tests, or field trials, where appropriate*

As the label claims are central to the efficacy evaluation for a biocidal product, it is important to understand exactly what is an efficacy claim, and be able to identify the individual components of a claim.

Label claims for biocidal products

As efficacy claims are assessed against the product 'when used normally in accordance with the conditions of authorisation', then it is important to define the 'normal use' of the product.

There are several pieces of information which will form part of the conditions of authorisation which relate to the efficacy assessment. These are:

1. The Formulation Type

This is determined by the product itself (e.g. a solvent based ready-for-use, a water based concentrate, a dusting powder, a gel bait, etc.).

2. Application Method

This is the method by which the product is intended to be applied (e.g. coarse spray, ultra-low volume (ULV) spray, bait station, skin lotion, etc.).

The application method may also describe a specific pattern of treatment. This is particularly common for spray applications, but may also apply to other formulation types. General descriptions of some common treatment patterns are given below.

(i) Surface treatments

These are treatments where the product is applied over surfaces such as walls, floors and ceilings, or as a treatment to outdoor surfaces. These treatments may involve treating a large area of surface or may only involve application to a narrow band.

Surface treatments can also include application to temporary or permanent bodies of water (e.g. in mosquito control) and to solid and semi-solid manure.

(ii) Crack and crevice treatments

These are treatments where products are applied into cracks and crevices where insects hide and harbourage, or through which they may enter the building. Such openings commonly occur at expansion joints, between different elements of construction and between equipment and floors. These openings may lead to voids such as hollow walls, equipment legs and bases, conduits and junction or switch boxes.

(iii) Contact (direct) spray treatments

These involve application directly onto insects, and are normally only possible when the insects are visible and available to be sprayed.

In practice this often restricts direct application methods to controlling flying insects (such as adult moths and houseflies), although some limited control of minor infestations of crawling insects (such as ants or beetles) may be possible.

(iv) Space treatments

These are treatments where the product is applied into the air rather than onto a surface.

They are intended to disperse small droplets or particles into the atmosphere of a room or other open space, where they will normally stay for a period of time (very small particles may stay in the air for several hours under still conditions).

(v) Spot treatments

These are treatments where products are applied to limited areas on which insect pests are likely to occur, but which will not be in contact with food or utensils and will not ordinarily be contacted by workers. These areas may occur on floors, walls and bases or undersides of equipment.

(vi) Baits

Bait treatments use products that are intended to be ingested by the target. This is normally through the insect feeding on the product directly, but may also include products which the target will come into contact with and later ingest during grooming/cleaning.

The attractiveness of these products is through the use of a palatable food base, however they may also incorporate an attractant (e.g. a pheromone) which is intended to attract the target pests over a greater distance.

3. Application Rate

This is the rate at which the product will be applied in use (e.g. apply 100 ml of product per square metre, apply at a rate of 1 bait station per 3 m^2, spray for 20 seconds, etc.).

For efficacy assessment purposes, it is useful to consider the application rate as the amount of *active substance* applied to surface area or volume.

Unlike a human health or environmental risk assessment which look at the maximum amounts of product which are considered to be acceptable (i.e. if the amount of active or

application rate increase, the risks to man or the environment will be unacceptable), an efficacy evaluation looks at the *minimum* application/dose rate which will be effective (i.e. if the application rate decreases, the product may not work).

4. Frequency of treatment and any specific interval between applications

Some products will be used in a way that will require more than one treatment. These products will give information on the treatment schedule which should be followed (e.g. insecticide re-treatment intervals or rodenticide re-baiting periods).

Together, these pieces of information define the 'normal use' of the product (e.g. a solvent based ready-for-use product to be applied as a coarse spray at a rate of 100 ml product m^{-2}), and efficacy must be demonstrated for the product when it is used in this way.

Whilst information on the application method and rate etc. will normally be clearly defined, the claims made for the effects of the product are much more difficult to identify.

5. Other specific conditions to be taken into account

Occasionally, the "normal use" of a product will involve the use of the product in conjunction with other activities. This will include the cleaning of an area prior to treatment. The contributions made by other components of an Integrated Pest Management procedure may also have to be taken into account.

Product labels and label claims

The product label is the major source of information on a product. It will give the use pattern to help determine the 'normal use' of the product, but will also make claims about the effectiveness of the product.

These label claims form the core of any efficacy evaluation. Efficacy is assessed mainly in relation to the claims made for the product. The norms and criteria set per insect pest will further guide the evaluation.

Whilst the phrase 'label claims' is generally used, this phrase actually encompasses all claims made for the product, not just those made on the label itself. Claims may also be made for a product with any accompanying information (such as leaflets) or on advertising material.

For efficacy purposes, all of these claims also have to be justified before they can be allowed onto a label.

What is a label claim?

A label claim is anything on the product label that makes a claim about what the product does or the benefits that will result from its use. At this moment there is no standard format for making claims about the effects and benefits of using the product, and the type and style of label claims can vary widely between different Member States.

For example, a product which claims to be 'For the control of cockroaches' in one Member State may claim that it 'Kills cockroaches fast!!' in another.

To aid in the evaluation process, a standardised method for identifying the main components of a label claim is set out below.

Label claims – understanding the components

A set of label claims will consist of 2 types of information which describe what the product will do when it is used (in accordance with its 'normal use'). These are:

1. The target species which the product will be effective against

   and

2. The effect (or effects) which the use of the product will have on the target species and the benefits which may result from this effect

Target species

The product label will give details about which species the product is to be used against. This information will often be quite specific (e.g. 'for the control of pharaohs ants' or 'kills ants, cockroaches, fleas and bed bugs or repels mosquitoes'). In these cases it is easy to identify what are the target species.

However there can also be instances where a more general claim is made, such as for use against 'crawling insects'. In these cases, it is difficult to require data on every crawling insect.

They will need to supply efficacy data on relevant representative species, which may be those used in standard test methods or those that the Applicant argues are representative of the use pattern of the biocide and the nature of the application (e.g. whether it is a space application or a surface application).

In some instances it is possible to allow a compromise on the label. For example, members of the general public may not know what species of fly is in their home, but the regulators will need to know what the product is effective against. In this particular instance it may be possible to allow a claim such as 'Effective against flying insects such as the housefly, mosquitoes and midges'.

The effects of using the product

The remaining parts of the label claim will describe the effects on the target organisms and benefits of using the product.

The major effects which are generally claimed are that a product will:

- kill, knock down, repel, attract, reduce the numbers of or inhibit a target organism
- control, reduce or prevent the build-up of a population
- prevent or reduce an undesirable effect.

For insecticide products, the following claims are the ones that are frequently encountered:

'Kill' claims generally refer to the death of an individual or a number of individuals (the death of an entire population is more generally found under a 'control' claim) and generally refer to an existing infestation.

'Knockdown' claims are generally restricted to insecticides and acaricides. A knockdown effect is one where a target insect becomes unable to carry out coordinated movement, but has not been killed.

Knockdown effects are often included in an insecticide product to produce a rapid, visible effect on a target in order to satisfy user expectations. These effects can be reversible, with insects able to recover after a period of time. Recovery is often dependent upon dose administered.

Knockdown claims may be found in conjunction with a kill claim, and many 'dual action' insecticide products contain two active substances - with one active substance producing a quick knockdown effect (such as a flying insect falling out of the air) whilst a second, slower acting, active substance produces the killing effect. Combined claims may be along the lines of 'knockdown within 10 minutes and kill within 2 hours'.

When it comes to efficacy testing, some companies use the two terms interchangeably, so you will get products or test reports mentioning 'knockdown' where a killing effect is actually meant. For evaluation purposes, knockdown and kill are considered to be separate effects.

'Complete control', 'colony kill' or *'nest kill'* claims will generally refer to the elimination of an entire infestation or population - i.e. use of the product will essentially 'remove the problem'.

As stated above, the mortality of individuals (rather than populations) is considered to be a 'kill' effect.

To highlight the difference between 'kill' and 'control', we can take the example of an ant nest outside of a house, close to the back door. The queen (which does all of the reproduction) remains hidden away in the nest and produces new ants for the colony, and the only ants seen outside of the nest are the sterile female workers.

An aerosol product which is intended to be sprayed onto ants wandering around in your kitchen to kill them will only be having a 'kill' effect. Killing off individuals or numbers of workers will have little effect on the nest and the colony as a whole, as the queen and fertile males will remain unaffected in the nest.

In order to remove the problem, you actually have to kill off the colony. So a product claiming to 'control' an infestation of ants would have to eliminate the queen or disrupt the ability of the colony to reproduce.

'Reduce' claims will generally refer to reducing the numbers of (but not completely eliminating) a target population. Whilst not eliminating an infestation may seem to be an odd claim to make, there are situations where it would be practically impossible to totally control a target population and where the best result is to reduce the scale of the problem.

An example of this would be reducing the fly burden in a poultry house or intensive animal house. However, the issue of resistance must always be kept in mind when considering treatments which do not fully control a population.

More complex label claims

Whilst a label claim is, at its most basic, a target and an effect, most claims are more complex, introducing further elements beyond the basic target/effect combination described above.

These additional parts of a label claim more fully describe the effects on the target organisms and benefits to be gained from using the product.

Claims for the effects and benefits of using the product can generally be broken down into 6 major components, which are described in Table 48.

The examples given in the table cannot be exhaustive, but are given to illustrate the type of information which appears in label claims.

Table 48: Components Making Up a Label Claim

Group		Label Claim
A	Target organism(s)	Against what target organism(s) will the product be used? • Specific insect (e.g. ants) • Several insects (e.g. ants and wasps) • General claim (e.g. flying and crawling insects)
B	Type of effect	What effect will the use of the product have on the target?

Group		Label Claim
		Examples include: • Kill • Knockdown • Control • Flushing • Attracting • Repelling
C	Time taken to produce the effect	How long will the product take to produce the effect? Examples include: • within 5 minutes • within 1 hour • within 3 months
D	Area of use	In what types of environment and on what type of surfaces will the product be used? For example: • indoors/outdoors • on hard porous and non-porous surfaces • on soft furnishings • in hospitals • in and around buildings
E	Duration of the effect	Will the product have a residual effect, and if so, how long for? For example: • for 6 weeks • for 3 months
F	User	Who can use the product? • Industrial use • Professionals • Consumers
G	Other specific claims	Does the product claim any other specific benefits? Examples include: • works against resistant species • helps prevent biting • protects fabric from damage

A label claim will not always contain all 7 components. For example, where no residual activity is being claimed, section E will not be represented, and where no specific other claims are being made, claims in section G will not be present.

The target organism (A), the type of effect (B) and area of use (D) and the user (F) should always be given.

On some labels, the time taken to product the effect (C) will not have been given (e.g. 'for the control of cockroaches') or is not a specific value (e.g. 'kills flies fast'). In these

cases, the evaluator will use the norms and criteria given per insect for the evaluation of the data.

Linking the components of the label claim

When initially trying to understand how the components of the label claims fit together, it can help to place the assorted claims into a table in order to identify how the various elements interact. For example:

Table 49: Example of linking label claims

Label claim (B)	Effect time (C)	Area of use (D)	Duration of effect (E)
Knocks down	within 5 minutes	- on hard porous and non-porous surfaces	for 6 weeks
Kills	within 1 hour	- on soft furnishings	

The beneficial effect of the product (B) will be accompanied by the timescale in which the effect will happen (C). In these cases, it must be demonstrated that the product will be efficacious within the stated time.

In the above example, it must be demonstrated that the product is capable of both knocking down the target insects *within 5 minutes* AND killing them *within 1 hour*.

The area of use (D) gives information about the conditions in which the product will be used and the type of surfaces it will be used on. The efficacy data supplied should demonstrate that the product will be efficacious in the areas specified or on representative surfaces of the types described.

In the example, it would have to be demonstrated that the product would produce its knockdown and kill effects *within the times stated* AND *on both hard surfaces and soft furnishings*.

The duration of effect (E) specifies the length of residual activity which must be demonstrated.

In the example, it must be demonstrated that the product is still capable of producing the effects on the specified surfaces 6 weeks after treatment (although not necessarily to the same degree as a fresh treatment).

Other claims can be linked into this process in the same way. For example, if claims were being made that the product was to be used against resistant individuals, then all of the above elements would have to be proved using a resistant test population to generate the data.

Once the various elements making up the label claims have been identified then the evaluation of the efficacy data submitted can proceed.

General guidance on the assessment of label claims is included in the paper "Broad principles of assessing efficacy in relation to claims made on the label for biocidal products", which was agreed at the Technical Meeting TM III 05 in October 2005, and at the subsequent CA meeting.

Guidance on type of and amount of data which would normally be required to support many of the major label claims is given for the main pest species elsewhere in this guidance.

Appendix 17. Species grid

Table 50: PT 18 Crawling Insects

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
1A	Flushing	Indoor	Crack & Crevice	"Flushes cockroaches out of hidden places"	*Blattella germanica* or *Periplaneta*	Data show Periplaneta flush before Blattella N.B This is true with pyrethroids, the case may be different with other actives. Fast acting pyrethroids may knockdown Blattella faster than they can be flushed, use Periplaneta in this case.	Any additional species need specific data.	Nymphs Adults
1B	Knockdown	Indoor	Direct Spray	"Knocks down cockroaches"; "Knocks down cockroaches in x seconds"	*Blattella germanica* and either *Periplaneta* species **or** *Blatta orientalis*	These species are representative of all domestic cockroaches found in Europe and around the world. Behavioural differences between species do not come into play when testing aerosols for direct spray efficacy.	We see little or no value in producing nymph/immature data in aerosol direct spray tests. Testing with only adults provides a very clear picture of product activity for registration studies. More than one life stage is an unnecessary burden.	Adults
1C	Kills	Indoor	Direct Spray	"Kills cockroaches"; "Kills cockroaches in x seconds"	*Blattella germanica* and either *Periplaneta* species **or** *Blatta orientalis*	See B.	See B	Adults
1D	Kills	Indoor	Direct Spray	"Kills ants"; "Kills in x seconds"	Lasius sp.	*Monomorium* ants are much smaller and more sensitive so would be covered by data for *Lasius*		Adults
1E	Kills	Outdoor	Direct Spray	"Kills ants"; "Kills in x seconds"	*Lasius* sp.			Adults

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
1F	Knockdown	Indoor	Direct Spray	"Kills crawling insects and other arthropods"	C + D and a variety of other common species e.g. *Forficula auricularia, Acheta domesticus, Cimex lectularius, Attagenus, Dermestes* sp., fleas, silverfish, booklice, carpet beetles, woodlice, ticks, centipedes, spiders	Multiple species are common world-wide. Test species will depend upon seasonal and local availability. See also B.	See B	Adults
1G	Knockdown	Indoor	Space spray; aerosols, gases, fogs, smokes	Knocks down crawling insects	Wood borers, carpet beetles, stored product beetles, other small crawling insects. Data required for claims on cockroaches (C) and fleas as surrogates for others			Adults, immatures
1H	Kills	Indoor	Space spray; aerosols, gases, fogs, smokes	Kills crawling insects	Wood borers, carpet beetles, stored product beetles, other small crawling insects. Data required for claims on cockroaches (3) and fleas			Adults, immatures and if claimed eggs
1I	Residual Kill	Indoor	Surface or Crack & Crevice Spray, Powders	"Kills cockroaches"; "Kills cockroaches up to x weeks or months"	*Blattella germanica* and either *Periplaneta* species **or** *Blatta orientalis*		Consider substrate and ageing period in the method	Adults and or immature stages. Specify realistic exposure period followed by reasonable "recovery" period.

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
1J	Residual	Indoor	Surface or Crack & Crevice Spray, Powder	Kills ants"; "Kills ants for x weeks or months"	*Lasius* sp. and/or *Monomorium pharaonis* as option (see 4)		Consider substrate and ageing period in the method	Adults Specify realistic exposure period followed by reasonable "recovery"
1K	Residual	Indoor	Surface or Crack & Crevice Spray, Powder	"Kills crawling insects and arthropods" ; "Kills for x weeks or months"	**K + L** and a variety of other common species e.g. *Forficula auricularia, Acheta domesticus, Cimex lectularius, Attagenus, Dermestes* sp., fleas, silverfish, booklice, carpet beetles, woodlice, ticks, centipedes, spiders		We propose only roaches be tested for full period.	Adults and immature stages. Consider substrate and ageing period in method. Specify realistic exposure period followed by "reasonable" recovery period.
1L	Residual	Indoor	Bait	"Kills cockroaches"; "Kills cockroaches for x weeks or months";	*Blattella germanica; Periplaneta americana* and *Blatta orientalis*		Either the claim is limited to a specific species or the three species are tested	Nymphs Adults. Consider ageing period in method. Provide harbourage and alternative food and water.
1M	Secondary kill	Indoor	Bait	"Kills cockroaches that do not visit the bait (secondary	*Blattella germanica; Periplaneta americana* and *Blatta orientalis*	Life stage to be tested depends upon a specific mode of action	Either the claim is limited to a specific species or the three species are tested	Life stage to be tested depends

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
				kill)"		(necrophagy versus coprophagy). Either nymphs or adults could be used.		upon a specific mode of action (necrophagy versus coprophagy). Either nymphs or adults could be used.
1N	Nest kill	Indoor	Bait	control of entire population of cockroaches	*Blattella germanica; Periplaneta americana* and *Blatta orientalis*		Either the claim is limited to a specific species or the three species are tested	Nymphs Adults
1O	Kill	Indoor	Bait	"Kills ants"; "Kills ants for x weeks or months";	*Monomorium pharaonis* and /or *Lasius niger*.		Either the claim is limited to a specific species or the two species are tested. Provide harbourage and alternative food and water.	Adults and all immature stages
1P	Colony kill	Indoor	Bait	"Kills the queen and the colony"	*Monomorium pharaonis* and /or *Lasius niger*.		Either the claim is limited to a specific species or the two species are tested. Provide harbourage and alternative food and water.	Adults and all immature stages. Use entire colonies including queens.
1Q	Kills	Indoor	Spray, powder	"Kills dust mites"	*Dermatophagoides* sp.			Adults and all immature stages, if claim include eggs.
1R	Residual Kill	Indoor	Spray, powder	"Kills dust mites for x weeks/months"	*Dermatophagoides* sp.		Consider substrate and ageing period in method. Specify realistic insect exposure period followed by reasonable "recovery" period.	Adults and all immature stages, if claim include eggs.

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
1S	Kill	Outdoor	Baits, Dusts, powders	Kills ants	*Lasius* sp.			
1T	Kill	Outdoor	Baits, Dusts, powders	"Kills the queen and the colony"	*Lasius* sp. and /or *Monomorium pharaonis*		Either the claim is limited to a specific species or the two species are tested. Provide harbourage and alternative food and water.	
1U	Kill	Outdoor	Sprays, liquid drenches	Kills ants	*Lasius* sp.		Add colony kill	
1V	Kill	Outdoor	Sprays, liquid drenches	"Kills the queen and the colony"	*Monomorium pharaonis* and /or *Lasius niger*.		Either the claim is limited to a specific species or the two species are tested. Provide harbourage and alternative food and water.	Whole colony
1W	Kill or repellent	Outdoor	**Physico-chemical barrier.** Installation between the soil and the future construction	**Preventive Pre- construction** treatment Prevent construction attack	All subterranean termites *Reticulitermes* sp. *Coptotermes* sp. *Heterotermes* sp.			
1X	Kill or repellent	Outdoor	**Chemical barrier** Injection in wall and soil	**Preventive Pre-construction** treatment Prevent construction attack	All subterranean termites *Reticulitermes* sp. *Coptotermes* sp. *Heterotermes* sp.			
1Y	Kill or repellent	Outdoor	**Chemical barrier** Injection in wall and soil	**Curative Post-construction** treatment	All subterranean termites *Reticulitermes* sp. *Coptotermes* sp. *Heterotermes* sp.			
1Z	Kill	Outdoor	**Baits system**	**Curative Post-construction**	*Reticulitermes sp.* *Coptotermes* sp.		Due to the specificity of baits, only species tested should be	

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
				treatment Colony elimination			claimed on the product label	
1AA	Kill	Indoor	Curative (Prevention is PT 8)	Kills dry wood termites	e.g. *Cryptotermes* sp.			
1AB	Barrier treatment	Indoor / Outdoor	Sprays, Powders	Prevents entry of crawling insects for x weeks or months	*Blattella germanica* and either Periplaneta species **or** *B. orientalis*, *Lasius* sp. See list above ("F") for selection, but expect roaches and ants to be the main claim			

Table 51: PT 18 Flying Insects

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
2A	Kills/ Knocks down	Indoor	Direct spray or room treatment	"Knocks down and/or Kills flies, mosquitoes";	*Musca domestica; Culex* sp. *or Aedes* sp.	These two species are representative of most urban species.	Flies and mosquitoes would be proxy insects for gnats and midges	adults
2B	kills	Indoor/ Outdoor	Aerosol, Coils, mats or liquid electrics; Plaquettes or similar devices	Kills mosquitoes for up to x hours	*Culex* sp. *or Aedes* sp.		All insects, for which claims are made, should be tested.	adults
2C		Outdoor	Nuisance flying insects (landfill area)	Kills "XYZ"	*Musca domestica* *Culex* sp. *or Aedes* sp.		All insects, for which claims are made, should be tested.	adults
2D		Outdoors	Direct and residual sprays	Kills "XYZ"	Claimed insects need to be tested			adult and larvae
2E		Indoor	Fumigants	Kills "XYZ"	Claimed insects need to be tested		All insects and insect stages for which claims are made,	Adults , eggs, and

	Action	SITE	APPLICATION METHOD	CLAIM	TEST SPECIES	RATIONALE	NOTES	INSECT STAGE
							should be tested.	larvae
2F	kills	Indoor	Direct spray or room treatment	"Kills flying moths"	*Plodia interpunctella* ***or*** *Tineola bisselliella*			adults
2G	kills	Indoor / Outdoor	Direct spray	"Kills wasps"	*Vespula* sp.			adults
2H	kills	Outdoor	Nest treatment (all methods)	"Kills wasp nests"; "Kills the queen"	*Vespula* sp. ***or*** *Dolichovespula* sp.		Test on whole nests	adults, queen for specific claim
2I	kills	Indoor	Closet or confined space treatments	"Kills clothes moths and larvae"; "Kills for x weeks or months"	*Tineola bisselliella*		All insects, for which claims are made, should be tested.	adults, eggs and / or larvae depending upon claim
2J	kills	Indoor	Baits	Kills "XYZ"flies	*Specifc species claimed on the label*			adults
2K	kills	Outdoor	Mosquitoes	Kills mosquito larvae	*Culex* sp. *Or Aedes* sp.		for IGRs the larval stage needs to be selected according to the mode of action.	last instar larvae
2L	kills	Indoor / Outdoor	Fly larvicides	Kills "XYZ"flies	*Specifc species claimed on the label*		for IGRs the larval stage needs to be selected according to the mode of action. Specify substrates)	last instar larvae

Appendix 18. List of currently available standard test methods for product type 18 insecticides/acaricides and product type 19 repellents/attractants (as far as they concern insects and other arthropods)

Recognised standard methods for the efficacy testing of biocidal products intended for the control of insects, acaricides and other arthropods.

This is a non-exhaustive list of available standard methods without distinction on suitability, usefulness, repeatability, the order of acceptability or robustness.

Table 52: General

Reference	Title	PT	Short description	Reference source
OPPTS 810.3000	General Considerations for Efficacy of Invertebrate Control Agents	18	General guide	US EPA
CEB 196		18	Test method to evaluate the efficacy of insecticidal bait products against common ant species	Vegephyl
EPPO pp1/152	Design and analysis of efficacy evaluation trials	18	This standard provides detailed advice on the design and analysis of efficacy evaluation trials. Primarily intended for use in plant protection but also very useful for biocides.	EPPO
EPPO pp1/181	Conduct and reporting of efficacy evaluation trials, including good experimental practice	18	This standard provides guidance on how to organize trials, and how to plan, conduct and assess them, then record and interpret them, so as to obtain comparable and reliable results. It is also based on the principle that trials should be performed according to Good Experimental Practice (GEP).	EPPO
EPPO Bulletin Volume 18, Issue 2, 1988 (p. 337-341)	EPPO Recommendations on fumigation standards	18		EPPO
OPPTS 810.3200	Livestock, poultry, fur- and wool-bearing animal treatments	18	This Product Performance Test Guidelines concerns efficacy testing of invertebrate control pesticides used on cattle, horses, sheep, goats, swine, chicken, turkeys, other domestic fowls, and fur-bearing animals, such as mink and rabbits.	US EPA
OPPTS 810.3300	Treatments to control pests of humans and pets	18	This guideline is concerned with efficacy testing of invertebrate control pesticides used on humans and pets.	US EPA
OPPTS 810.3500	Premises treatments	18	This guideline provides recommendations for the design	US EPA

Reference	Title	PT	Short description	Reference source
			and execution of laboratory and field studies to evaluate the performance of products applied in or around premises.	
SANS 5233	Pesticides - Biological evaluation of mists and fogs	18	This standard specifies a method for the biological evaluation of the efficacy of pesticidal mists and fogs.	SABS
SANS 5576	Pesticides – Biological evaluation of insecticidal oil-based space spray in low-pressurized dispensers	18	This standard specifies a method for the determination of insecticidal oil-based space sprays in low-pressurized dispensers.	SABS
SANS 5583	Pesticides – Biological evaluation of the contact efficacy of liquid residual insecticides	18	This standard describes a method for conducting biological contact efficacy tests of liquid residual insecticides (including wettable powders and water dispersible granules).	SABS
SANS 6136	Pesticides – Biological evaluation of materials that release an insecticide upon heating	18	This standard describes the method for conducting biological efficacy tests on materials that release insecticides on heating.	SABS
SANS 5689	Pesticides – Biological evaluation of the direct spray knockdown and killing proprieties of liquids and aerosol dispensers	18	This standard describes a method for conducting biological evaluation tests of liquid and aerosol formulations.	SABS
SANS 5690	Pesticides - Biological evaluation of the properties of solid fly baits	18	This standard describes a method for conducting biological evaluation tests on solid fly baits.	SABS
SANS 5807	Rooms, conditions, equipment,	18	This standard specifies the test rooms, test conditions,	SABS

Reference	Title	PT	Short description	Reference source
	and insects and their handling, for use in pesticide testing		test equipment, test insects and their handling, for use in pesticide testing.	
SANS 899	Pesticides - Insecticidal space spray in pressurized aerosol dispensers	18	This standard covers requirements for insecticidal space sprays in pressurized aerosol dispensers and is intended for use in food-handling, food-processing and catering establishments.	SABS
CTD/WHOPES/IC/96.1	Evaluation and testing of insecticides	18	Report of the WHO Informal Consultation on the evaluation and testing of insecticides, WHO, Geneva, 7-11 October 1996	WHO

Table 53: Crawling Insects: Cockroaches

Reference	Title	PT	Short description	Reference source
CEB 249		18	Method for testing the efficacy in the laboratory and under practical conditions of use, of insecticidal baits intended for the control of cockroaches in premises	Vegephyl
CEB 159		18	Trial method to evaluate the efficacy of insecticidal products for the control of cockroaches in buildings under practical conditions	Vegephyl
ENV/JM/MONO(2013)3	Guidance Document on Assays for Testing the Efficacy of Baits against Cockroaches	18	Outlines methods available for testing efficacy and effectiveness of baits against cockroaches.	OECD
SANS 5458	Pesticides – Rearing and handling of the German cockroach (*Blatella germanica*	18	This standard specifies a method for the rearing and handling of the German cockroach (*B. germanica (L.)*.	SABS

Reference	Title	PT	Short description	Reference source
	(L.))			
WHO/VBC/75.593	Instructions for determining the susceptibility or resistance of cockroaches to insecticides	18	The purpose of this test is to detect the presence of resistant individuals in a mosquito larval population.	WHO

Table 54: Crawling Insects: Termites

Reference	Title	PT	Short test description	Reference source
CTBA-BIO-E-007	Evaluation of the anti-termite efficacy of a barrier placed in an alkaline medium.	18	Laboratory test method to assess the loss of efficacy that an alkaline medium could induce on an anti-termite barrier.	FCBA
CTBA-BIO-E-008/2	Evaluation of the anti-termite efficacy of a physico-chemical barrier - Field test - Device without concrete slab.	18	This test method presents the different steps to evaluate the effectiveness of an anti-termite protective film in an outdoor environment.	FCBA
CTBA-BIO-E-016	Exposure of anti-termite physico-chemical barriers to solar radiation.	18	This test method presents the principles that must be applied to expose plastic materials to solar radiation. It is applicable to plastic-based physico-chemical termite barriers.	FCBA
FCBA-BIO-E-041		18	Efficacy criteria for performance of CTBA-BIO-E-xx and FCBA-BIO-E-xx tests	FCBA
FCBA-BIO-E-045		18	This method is designed to test the preventive treatment of physico-chemical barrier with a concrete slab. All the efficacy tests performed with CTBA-BIO-E-008 remain valid.	FCBA

Reference	Title	PT	Short test description	Reference source
FCBA-BIO-E-053		18	This method replaced CTBA-BIO-E-001 and CTBA-BIO-E-002. All the efficacy tests performed following CTBA-BIO-E-001 and CTBA-BIO-E-002 methodologies remain valid.	FCBA
NF X 41-542	Wood preservatives - Anti-termite treatment product for floors, walls, foundations and masonry - Accelerated aging test of treated materials before biological tests - Percolation test.	8+ 18	Laboratory test method to determine the efficacy against termites of products or material used as barrier designed for ground and/or wall.	AFNOR
NF X 41-543-1	Wood preservatives - determination of the efficacy of a bait-trap system - part 1: Efficacy of the insecticide formulation - laboratory method.	8+ 18	This test method is applicable to sustained insecticidal formulations intended for use in bait trap systems.	AFNOR
NF X 41-543-2	Wood preservatives - determination of the efficacy of a bait-trap system - part 2: field method.	8+ 18	This test method is intended to evaluate the efficacy of the baits in an experimental site where termite activity is reported. Consumption of the tested bait must be registered at least in the first 6 months after the introduction of the baits. The elimination of termites in the experimental site should be registered maximum after 18 months (counted since the introduction of the first tested bait), excluding the winter period.	AFNOR
NF X 41-543-3	Wood preservatives - determination of the efficacy of a bait-trap system - part 3: performance criteria.	8+ 18	Applies to anti-termite products with a delayed effect intended for use in an identified bait trap system and the methods of use of which are known. It defines the criteria to be achieved in the tests described in part 1 and part 2.	AFNOR

Reference	Title	PT	Short test description	Reference source
NF X 41-550	Termites - Determination of the effectiveness against termites of products or materials used as barrier designed for ground and/or wall - Laboratory method	8+ 18	It describes a test method for determining the effectiveness of a product or material intended to constitute a protective barrier for constructions against subterranean termites. To characterize the persistence of the efficacy of these products and materials, this method can be applied following an aging test.	AFNOR
NF X 41-551	Termites - Determination of the effectiveness against termites of products or material used as barrier designed for ground and/or wall- Performance criteria	8+ 18	It defines the criteria for the effectiveness of products or materials intended to constitute a protective barrier for buildings against subterranean termites.	AFNOR
OPPTS 810.3800	Methods for efficacy testing of termite baits	8+ 18	This test method concerns the product performance testing for evaluation of products used as baits to kill and control termites.	US EPA

Table 55: Crawling Insects: Other Crawling Insects

Reference	Title	PT	Short test description	Reference source
AATCC 194	Test Method for Anti-House Dust Mite Properties of Textiles under Long-Term Test Conditions	18	This test method is for the evaluation of the degree of anti-house dust mite activity in a long-term testing environment for textiles treated at the manufacturing level for this purpose.	AATCC
OCSPP 810.3900	Product Performance Test Guidelines; OCSPP 810.3900 Laboratory Product Performance Testing Methods for Bed Bug Pesticide Products	PT 18	This guideline provides recommendations for the design and execution of laboratory test to evaluate the performance of products intended to repel, attract, and/or kill the common bed bug (*Cimex lectularius*).	US EPA

Reference	Title	PT	Short test description	Reference source
OPPTS 810.3100	Soil treatments for imported fire ants	18	This guideline contains recommended test methods for evaluating the performance of products for the treatment and control of imported fire ants.	US EPA

Table 56: Flying Insects

Reference	Title	PT	Short test description	Reference source
CEB 107	Method for testing the practical effectiveness of insecticidal products intended for the control of barn flies in premises for keeping domestic animals	18	Trial method to evaluate the efficacy of insecticidal products for the control of stable flies in premises for the rearing of domestic animals under practical conditions	Vegephyl
MS 1911, part 1	Household insecticide products - evaluation method for biological efficacy - part 1: glass chamber method	18	This standard specifies a method for the evaluation of the biological efficacy of household insecticide products using the glass chamber method. This test method provides a satisfactory means to determine the relative effectiveness of common household insecticide products, namely mosquito electric vapourising liquid, mosquito vapourising mat and mosquito coils. The method is suitable for testing the above insecticide products against mosquitoes.	JSM
MS 23	Household insecticide products - Mosquito coil - Chemical, physical and biological efficacy requirements (Fifth revision)	18	This Malaysian Standard specifies the minimum chemical and physical requirements, and biological efficacy of mosquito coil products intended for household use against mosquitoes.	JSM
OPPTS 810.3400	Mosquito, black fly, and biting midge (sand fly) treatments	18	Test of insecticides against flying insects: Mosquito, Black Fly and Biting Midge (Sand Fly)	US EPA

Reference	Title	PT	Short test description	Reference source
US CSMA Aerosol Guide, 7 th Edition, (1981), p. 129-134	Test method for aerosol space sprays against flying insects	18	Test of insecticides against flying insects:	CSMA
WHO/VBC/81.812	Mosquito larvae resistance to insect development inhibitors	18	Test kit and instruction sheet	WHO
WHO/VBC/81.806	Instructions for determining the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides	18	Test kit and instruction sheet	WHO
WHO/VBC/81.807	Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides	18	Test kit and instruction sheet	WHO
WHO/VBC/81.811	Blackfly larvae	18	Test kit and instruction sheet	WHO
WHO/VBC/81.813	Houseflies, tsetse flies, stable flies, blowflies, etc.	18	Test kit and instruction sheet	WHO
WHO/CVB/81.5	Test kit for bioassays on wall surfaces	18	Test kit and instruction sheet	WHO
WHO/CDS/CPC/MAL/98.12	Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces	18	Report of the WHO informal consultation	WHO
WHO/CDS/WHOPES/GCDPP/2003.5	Space spray application of insecticides for vector and public health pest control – a practitioner's guide	18	Brief description of the main types of space spray equipment as well as the operational guidelines for space spray application of insecticides.	WHO

Reference	Title	PT	Short test description	Reference source
WHO/CDS/WHOPES/GCDPP/2005.13	Guidelines for laboratory and field testing of mosquito larvicides	18	This document provides specific and standardized procedures and guidelines for testing larvicides, including bacterial larvicides and insect growth regulators against mosquitoes.	WHO
WHO/CDS/NTD/WHOPES/GCDPP/2006.3	Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets	18	This document provides specific and standardized procedures and guidelines for testing mosquito adulticides for indoor residual spraying and for treatment of mosquito nets.	WHO
WHO/HTM/NTD/WHOPES/2009.2	Guidelines for efficacy testing of insecticides for indoor and outdoor ground-applied space spray applications	18	The document provides guidance and stepwise procedures on laboratory studies, field testing and evaluation leading to the determination of efficacy, and application rates of insecticides for operational use in indoor and outdoor ground-applied space spray applications. With some modifications the guidelines can be used to determine efficacy against other flying vectors and pests.	WHO

Table 57: Insecticides Against Textile and Stored Product Pests

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Type of Reference Source
CEB 135 BIS		18	Method for the laboratory study of the efficacy of insecticide preparations intended for the treatment of industrial processing storage premises and the marketing of products of animal or plant origin.	Vegephyl
CEB 213		18	Method for studying the effectiveness of a fumigant for the disinsection of premises for the storage, processing and production of foodstuffs.	Vegephyl
CEB 224		18	Method for studying the efficacy of fumigants for the disinsection of stored foodstuffs.	Vegephyl

Reference	Title	PT	Short test description (if test method available or information provided from elsewhere)	Type of Reference Source
PP1/201	Fumigants to control insect and mite pests of stored plant products	18 + 20		EPPO
PP1/202	Space and structural treatments of store rooms	18		EPPO
PP1/203	Admixture of plant protection products to stored plant products to control insects and mites	18 + 20		EPPO
PP1/204	Laboratory testing of plant protection products against insect and mite pests of stored plant products	18		EPPO
NF G39-011	Properties of textiles - Textiles and polymeric materials having anti-dustmite activity - Characterisation and measurement of anti-dustmite	18	This document describes a general method for determining the anti-mite activity of textiles and polymeric materials. The method is applicable to all textiles and polymeric materials with anti-mite activity, except those whose structure does not allow the contact defined under the test conditions.	AFNOR
NF ISO 3998	Textiles- Determination of resistance to certain insect pests	18	This standard specifies a method for the determination of the resistance of textiles to the larvae of certain insects.	AFNOR
ISO 3998	Textiles - Determination of resistance to certain insect pests	18	Applicable to all textiles containing animal fibres in any proportion. Comparing the resistant material against a non-resistant material.	ISO

Table 58: Repellents and Attractants

Reference	Title	PT	Short test description	Reference source
Ctgb	Evaluation manual for the authorization of biopesticides according to regulation (EC) No. 1107/2009, microorganisms, botanicals, semiochemicals.	19	This document describes in more detail the data requirements and risk assessment for biopesticides.	Ctgb
CVMP/411/2001	Specific efficacy requirements for ectoparasiticides in sheep	19	This document provides guidance on the study of the efficacy of products against the principal parasites found in sheep. It can be applied also to products against less common (regional) ectoparasites, providing that any adjustments to the methods are justified.	EMA
CVMP/625/2003	Specific efficacy requirements for ectoparasiticides in cattle	19	This document provides guidance on how to study the efficacy of products in cattle against all arthropod species that need animal involvement for completing their life-cycle.	EMA
EMEA/CVMP/EWP/005/2000-Rev.3	Guideline for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats	19	This guideline provides specific guidance with respect to the testing and evaluation of efficacy of veterinary antiparasitic products that are intended for the treatment and prevention of tick and flea infestations in dogs and cats, and includes information for the testing of veterinary systemically and locally acting antiparasitic products and products containing substances with insect growth regulating properties (IGRs), either as mono-preparations or in combination with an adulticide.	EMA
EN 152	Wood preservatives - Determination of the protective effectiveness of a preservative treatment against blue stain in wood in service - Laboratory method		This European Standard specifies a method which is only suitable for testing preparations and systems which are intended to prevent the occurrence of blue stain fungi in wood in service. This European Standard lays down a method for determining the effectiveness of a preparation applied by e.g. brushing, spraying, spraying tunnel, dipping or vacuum and pressure treatments resulting in an equivalent retention of product in preventing the	CEN

Reference	Title	PT	Short test description	Reference source
			development of blue stain fungi in wood in service.	
EN 14360	Protective clothing against rain - Test method for ready-made garments - Impact from above with high energy droplets	19	This European Standard specifies a test method for determining the rain tightness of clothing for protection against rain, using a static manikin exposed to artificial rain. It is applicable to the testing of jackets, trousers, coats and one or two piece suits.	CEN
ISO 6330	Textiles - Domestic washing and drying procedures for textile testing	19	This standard specifies domestic washing and drying procedures for textile testing. The procedures are applicable to textile fabrics, garments or other textile articles which are subjected to appropriate combinations of domestic washing and drying procedures.	ISO
ENV/JM/MONO(2000)7	Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation	19	The purpose of this guidance is to apply the principles of the Three Rs (Replacement, Reduction, Refinement) to the use of animals in regulatory toxicity tests	OECD
ENV/JM/MONO(2001)12	Guidance for Registration Requirements for Pheromones and Other Semiochemicals Used for Arthropod Pest Control	19		OECD
ISBN 978-1-891127-75-5	Atlas of Stored-Product Insects and Mites	19		AACC International
OPPTS 810.3300	Treatments to control pests of humans and pets		This guideline is concerned with efficacy testing of invertebrate control pesticides used on humans and pets.	US EPA
PP1/152	Design and analysis of efficacy evaluation trials	19	This standard is intended to provide general background information on the design and analysis of efficacy evaluation	EPPO

Reference	Title	PT	Short test description	Reference source
			trials.	
PP1/181	Conduct and reporting of efficacy evaluation trials, including good experimental practice	19	This standard provides guidance on how to organize trials, and how to plan, conduct and assess them, then record and interpret them, so as to obtain comparable and reliable results.	EPPO
PP1/264(2)	Principles of efficacy evaluation for mating disruption pheromones	19	This standard describes the general principles of trial design for the efficacy evaluation of mating disruption techniques based on pheromones. These techniques are based on female sex pheromones, but others could also be used, e.g. aggregation pheromones which attract both sexes for mating or, in rare cases, where males produce sex pheromones.	EPPO
PP1/296	Principles of efficacy evaluation for low-risk plant protection products	19	The objective of this document is to provide a framework for the minimum efficacy data requirements needed to demonstrate that a low-risk plant protection product is sufficiently effective (and crop safe) for authorization.	EPPO
RIVM report 090013003/2014	General Fact Sheet, General default parameters for estimating consumer exposure - Updated version 2014	19	The document contains default values for the room in which the exposure takes place and for the person that is exposed. In addition, it presents information on the ventilation in houses, inhalation rates and data on activity patterns.	RIVM
SANCO/11470/2012-rev. 8	Guidance document on botanical active substances used in plant protection products	19		DG SANCO
SANTE/12815/2014 rev. 5.2	Guidance document on semiochemical active substances and in plant protection products	19		DG SANTE
Alcaine-Colet A. et al.	Rearing the scuttle fly *Megaselia scalaris* (Diptera: Phoridae) on	19		PeerJ 3

Reference	Title	PT	Short test description	Reference source
	industrial compounds: implications on size and life-span			(1526): e1085
Arnault I. et al.	Efficiency comparison of three attractant products against webbing clothes moth *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae) using an adapted four arms olfactometer	19		Julius-Kuhn-Archiv, 425
Beerwinkle K.R. et al.	Free-choice olfactometer bioassay system for evaluating the attractiveness of plant volatiles to adult *Helicoverpa zea*	19		Southwestern entomologist 21(4): p. 395-405
Benoit J.B. et al.	Addition of Alarm Pheromone Components Improves the Effectiveness of Desiccant Dusts Against *Cimex lectularius*	19		Journal of Medical Entomology, 46(3), p. 572-579
Blume R.R. et al.	Tests of aerosols of deet for protection of livestock from biting flies	19		Journal of economic entomology 64(5): p. 1193-1196
Büchel, K., Kleier S., Dautel, H.	Minimizing animal testing using highly standardized laboratory bioassay for repellent against cat fleas	19		29th Annual Meeting of the German Society for Parasitology, Bonn 2021

Reference	Title	PT	Short test description	Reference source
Büchel K. et al.	Repellent efficacy of DEET, Icaridin, and EBAAP against *Ixodes ricinus* and *Ixodes scapularis nymphs* (Acari, Ixodidae)	19		Ticks and Tick-borne Diseases 6(4): p. 494-498
Carroll J.F. et al.	Comparative Activity of DEET and AI3-37220 Repellents Against the Ticks *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae) in Laboratory Bioassays	19		Journal of Medical Entomology 41(2): p. 249-254
Carroll S.P.	Prolonged Efficacy of IR3535 Repellents Against Mosquitoes and Blacklegged Ticks in North America	19		Journal of Medical Entomology 45(4): p. 706-714
ECDC	Rapid risk assessment: Autochthonous cases of dengue in Spain and France	19		ECDC
Dautel H et al. International Journal of Medical Microbiology, Vol 293, Supplement 37, April 2004, pages 182-188	A novel test system for detection of tick repellents	19	The so-called Moving Object Bioassay is described, a tool for testing the strength of potential tick repellents quantitatively. Endpoint measured is the attachment rate of *Ixodes* ticks.	International Journal of Medical Microbiology
Dweck H. et al.	Olfactory Proxy Detection of Dietary Antioxidants in Drosophila	19		Current Biology 25(4): p. 455-466
Fradin M., Day J.	Comparative efficacy of insect	19	Human subjects: Arm in cage studies (15 volunteers, 10 mosquitoes (*Aedes aegypti*) in each cage. Endpoint: elapsed	The New England

Reference	Title	PT	Short test description	Reference source
	repellents against mosquito bites		time to first bite. Category of protection A-H (significantly different mean complete protection time; ANOVA & Tukey's). No need to recalculate the results to "real condition" (simulate real condition)	Journal of Medicine 347(1): p. 13-18
Geier M., Boeckh J.	A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours	19		Entomologia Experimentalis et Applicata 92(1): p. 9-19
Govere J., Durham D.	Techniques for Evaluating Repellents	19		Insect Repellents book: p.147-160, CRC Press
Herholz C. et al.	Efficacy of the repellent N,N-diethyl-3-methyl-benzamide (DEET) against tabanid flies on horses evaluated in a field test in Switzerland	19		Veterinary Parasitology 221: p. 64-67
Hill J.A., Robinson P.B., McVey D.L., Akers W.A. et al.	Evaluation of mosquito repellents on the hairless dog	19		Mosquito news 39(2)
Ja W.W. et al.	Prandiology of Drosophila and the CAFE assay	19		Proceedings of the National Academy of Science, 104(20): p. 8253-8256

Reference	Title	PT	Short test description	Reference source
Japin, M. and Haanen, G. A. Y.	Culicoides species in the Netherlands: a comparison between tent traps and an Onderstepoort black light trap and the effect of an insect blanket on the biting rat	19	The aims of the present study were to determine which species in which numbers of Culicoides (that potentially serve as vectors for AHSV) are attracted to horses in the Netherlands and to compare these results with the Culicoides species and numbers caught in the Onderstepoort black light trap during the same period. The second aim was to evaluate the use of an insect blanket on the biting rate of Culicoides species.	Faculty of Veterinary Medicine Theses (2013) https://dspace.library.uu.nl/handle/1874/285251
Kline D.L., Mann M.O.	Evaluation of butanone, carbon dioxide, and 1-octen-3-ol as attractants for mosquitoes associated with North Central Florida bay and cypress swamps	19		Journal of the American Mosquito Control Association 14(3): p. 289-297
Knaden M. et al	Spatial Representation of Odorant Valence in an Insect Brain.	19		Cell 1(4): p. 392-399
Krüger, A., S. Knobelspieß, and E. Schmolz	Development and evaluation of testing methods for ant repellents	19	This document describes two test systems for efficacy evaluation of ant repellents with the substances DEET 50%, Margosa extract 100%, baking powder: sodium hydrogen carbonate and sea sand. They are designed as choice tests and allow testing of solid and liquid substances.	9th International Conference on Urban Pests, Birmingham. 2017
Marchiondo A.A., et al.	World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition: guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of	19	These guidelines are intended to assist the planning and conduct of laboratory and clinical studies to assess the efficacy of ectoparasiticides applied to dogs or cats for the purpose of treating, preventing and controlling flea and tick infestations.	Veterinary Parasitology, 194(1): p. 84-97

Reference	Title	PT	Short test description	Reference source
	flea and tick infestations on dogs and cats			
Mottet et al.	Effectiveness of stable fly protectants on adult horses	19		Journal of Equine Veterinary Science 69: p. 11–15
Moreno-Gómez M. et al.	From the field to the laboratory quantifying outdoor mosquito landing rate to better evaluate topical repellents	19	This study aimed to estimate the landing rate outdoors, in an area of Europe highly infested with the Asian tiger mosquito, Aedes albopictus, and to determine how to replicate this rate in the laboratory. This study provides useful reference values that can be employed to design new evaluation standards for topical repellents that avoid field conditions given that the latter exposes study participants to health risks.	Journal of Medical Entomology; tjaa298
Moreno-Gómez M. et al.	Two new alternatives to the conventional arm-in-cage test for assessing topical repellents	19	Two alternative laboratory methods that use mosquito landing rates more representative of those in the field were assessed. These methods showed to be potential alternatives to the current AIC method, as well as being a better proxy for recreated field mosquito landing rates., reduced variability among study participants, and achieved reproducible protection times across laboratories.	Journal of Medical entomology; tjab050
Mulatier M. et al.	DEET Efficacy Increases With Age in the Vector Mosquitoes *Anopheles gambiae* s.s. and *Aedes albopictus* (Diptera: Culicidae)	19		Journal of Medical Entomology 55(6); p. 1542-1548
Obermayr U. et al.	A novel test cage with an air ventilation system as an alternative to	19		Journal of Medical

Reference	Title	PT	Short test description	Reference source
	conventional cages for the efficacy testing of mosquito repellents			Entomology 47(6): p. 1116-1122
Okumu F. et al.	Using nylon strips to dispense mosquito attractants for sampling the malaria vector *Anopheles gambiae* s.s.	19		Journal of Medical Entomology 47(2): p. 274-282
Plarre R. et al.	Effects of oil of cloves and citronellol, two commercially available repellents, against the webbing clothes moth *Tineola bisselliella* Hum. (Lepidoptera: Tineidae)	19		Journal of Pest Science 70(3): p. 45.
Smith C.N. et al.	Factors Affecting the Protection Period of Mosquito Repellents	19		U.S. Department of Agriculture
Stensmyr M. C. et al.	A Conserved Dedicated Olfactory Circuit for Detecting Harmful Microbes in Drosophila	19		Cell 151(6): p. 1345-1357
Hummel, E., Kleeberg, H. 1997. in: Practice orientated results on use and production of Neem-Ingredients and Pheromones V. Proceedings of the 5th workshop, Wetzlar,	Effect of the neem extract formulation neemazal-t/s on the green pea aphid acyrthosiphon pisum in the laboratory (1995), in: Practice orientated results on use and production of Neem-Ingredients and Pheromones V	19		Trifolio-M GmbH

Reference	Title	PT	Short test description	Reference source
Germany, January 22-25, 1996				
Vander Pan A. et al.	A Novel Simulated-Use Test for Determining the Efficacy of Insecticides Against Bed Bugs (Hemiptera: Cimicidae)	19	The objective of this study was to develop a simulated-use test system for efficacy testing of insecticides with residual properties against bed bugs, imitating a typical insecticide barrier treatment under practical conditions.	Journal of Economic Entomology 112(5), p. 2345-2353
Vythilingam I. et al.	Evaluation of carbon dioxide and 1-octen-3-ol as mosquito attractants	19		The Southeast Asian Journal of Tropical Medicine and Public Health 23(2): p. 328-331
Wade S.E., Georgi J.R.	Survival and reproduction of artificially fed cat fleas, *Ctenocephalides felis Bouché* (Siphonaptera: Pulicidae)	19		Journal of Medical Entomology, 25(3)p. 186–190,
Wang C. et al.	Repellency of selected chemicals against the bed bug (Hemiptera: Cimicidae)	19		Journal of Economic Entomology, 106(6) 2013: p. 2522-2529
SANS 5695	Pesticides – Biological properties - Efficacy of mosquito repellents	19	Specifies 3 methods for the biological evaluation of the efficacy of mosquito repellents.	SABS

Reference	Title	PT	Short test description	Reference source
OPPTS 810.3700	Insect repellents to be applied to human skin	19		US EPA
WHO	Guidelines for efficacy testing of spatial repellents	19	The document provides guidance and describes steps for laboratory testing and for semi-field and field evaluations of spatial repellent products (technical materials and formulated products) designed to provide protection in a specific space (indoor and/or outdoor) against mosquitoes.	WHO
WHO/HTM/NTD/WHOPES/2009.4	Guidelines for efficacy testing of mosquito repellents for human skin	19	The purpose of these guidelines is to provide specific and standardized procedures and criteria for efficacy testing and evaluation of mosquito repellents for human skin. Their aim is to harmonize the testing procedures carried out in different laboratories and institutions in order to generate comparable data for registering and labelling such products by the national regulatory authorities.	WHO
MS 1497	Household insecticide products - personal mosquito repellent - evaluation method for biological efficacy	19	Methods of biological evaluation of the efficacy of repellent - bioassay method for mosquito repellent on human skin	Malaysian Institute of Chemistry
7AE17a	Demonstration of Efficacy of Ectoparasiticides	19	This document provides general requirements for the assessment of efficacy of an ectoparasiticide preparation, containing novel or established active ingredients.	EMA

Appendix 19. Efficacy guideline with Cockroach; field trial

This guidance describes an example of a field trial to determine efficacy of a product against the German cockroach (*Blattella germanica*).

Global design

In a pre-test it is established whether the population of cockroaches in an object is large enough for a field trial. An indication of the population size is obtained in the pre-test by using a spray with expelling action or by setting glue traps.

If the population size is large enough, a pest control operation is performed. The efficacy of the product is determined by measuring the population size again 8 weeks later and comparing it to the initial value.

During these 8 weeks the effect of the control operation should be checked at least 4 times at regular intervals (possibly using glue traps). The investigator himself should perform these checks during the trial.

Requirements for the practical use situation in order to be suitable as test object.

The field trial is performed in three separate objects.

Recommendations for the practical use situation to produce a good field trial for control of the German cockroach are as follows:

1. History of insecticide use should be described with as much detail as possible (which product, active ingredient, when …). Object with recent insecticide use should not be included in the test.
2. The test object should preferably and where possible be hermetically sealed off from the surrounding buildings. If there are adjacent buildings, all cracks and crevices on the outside of the test object should be treated with an authorised biocidal product with residual action.
3. The test object should preferably contain at least a kitchen or kitchen unit, with one or more refrigerators or freezers.
4. Cockroaches should be present in the test object, both in the kitchen or kitchen unit as elsewhere.
5. In the preceding 8 weeks no other chemical control of cockroaches should have taken place in the test object.

Field trial

The pre-test

Aim: To determine whether the population is large enough for a field trial.

Execution: Within 1 week before the control operation.
The pre-test can be conducted in two different ways.

1. By using a spray liquid with an expelling action (e.g. pyrethrins):
   Spray under the refrigerator and one other place in the kitchen where there are probably many cockroaches.
   Spray for 3 seconds and count the cockroaches that emerge during 1 minute.
2. By using glue traps
   Place glue traps at places where many cockroaches are expected.
   Number per unit area: 5 glue traps per 100 m^2
   Describe clearly where the glue traps are placed, and record the number of trapped cockroaches after an appropriate period, usually either overnight, or

after up to 3 days (e.g. weekend), depending upon the scale of the infestation (shorter trap periods for heavier infestations to avoid traps becoming saturated and failing to catch cockroaches later during the monitoring period; longer periods when infestation level is low and few cockroaches are trapped each night).

Criteria for a suitable test object

- When a trap is placed for 48 hours in the kitchen or in the kitchen unit behind the refrigerator, it should contain at least 10 adult cockroaches at the end of this time, as well as several nymphs.
- Several cockroaches should be caught on at least one glue trap, which is placed at another place in the kitchen or kitchen unit and on one trap, which is placed outside the kitchen or kitchen unit, within 48 hours.

Or

- When using a spray with expelling action, at least 5-10 cockroaches per sprayed site should be counted.

The test

Duration of the control period until measurement of efficacy is about 8 weeks.

The pest control is performed according to the directions for use of the product.

During these 8 weeks the investigator will check the progress of the control at least 4 times.

Directions for use of an insecticide in the form of a spray liquid:

- It should be clear how much product is used, on average 1 L/20 m^2 is sprayed;
- Treatment of cracks and crevices should be done where necessary;
- If stated on the label, a second treatment can be performed.

Directions for use of an insecticide in the form of a powder:

- It should be clear how much product is used.

Directions for use of an insecticide in the form of bait:

- Number of baits placed per unit area should be according to directions for use;
- Precise descriptions of where the baits are placed should be given;
- The baits that are placed remain *in situ* for 8 weeks continuously, unless stated differently on the label.

Required results

At least 4 times during the test and at the end of the test (about 8 weeks after the start), an estimate of the population size is obtained in the same manner as during the pre-test. The difference in population size before and 8 weeks after the control operation provides the degree of efficacy of the product.

Appendix 20. Current Antifouling Coatings

The current major types of antifouling coatings are outlined below, together with a brief description of their properties. This list is not exhaustive, and product applications may not fall within these categories. Applicants may submit novel coating types not covered by this list.

Table 59: Current Antifouling Coatings

Coating Type	Description, mode of action and properties
Soluble matrix	In coatings of this type the active substance(s) has (have) been physically mixed ('freely associated') into a resin matrix. Upon exposure to seawater the slightly acidic matrix slowly dissolves releasing the active substance(s) into the water. (Seawater is slightly alkaline (pH 8) and the acidic matrix dissolves). Continuous dissolution of the coating surface will occur resulting in fresh actives being released until eventually the film is exhausted. Soluble matrix antifouling products typically show a biocide release rate curve which decays exponentially. The soluble matrix coatings have reduced mechanical properties that limit their film thickness. The paint film thickness of these coatings depletes over time in a fairly imprecise manner and the film does not show smoothing characteristics on ships in service. Such coatings are normally specified for lifetimes of typically 12-36 months.
Insoluble matrix	This type of coating contains a mixture of resins that together form an insoluble binder phase. One or more active substances are physically mixed into this matrix. As seawater enters the paint film, the biocides are released by dissolution and diffusion from within the insoluble matrix. After active substance have been released from the film, the binder remains intact and an empty 'honeycomb' structure (the leached layer) remains at the paint surface. This type of coating has a high initial release rate, which decreases exponentially with time as the active substance(s) have further distance to travel through the paint film. The rate of diffusion of biocide from within the film then becomes a limiting factor in maintaining an effective biocide release rate and hence preventing fouling. Insoluble matrix antifouling coatings do not show film-depletion or polishing as the resin is insoluble. The biocide release process continues until exhaustion of the coating. The higher mechanical strength obtained with these coatings allows for applications of thicker systems and coating lifetimes of typically 12- 36 months are attainable.
Self-polishing	This group is currently the most common and covers a range of different technologies that deliver the active substance through a gradual depletion/ablation of the paint film throughout the lifetime of the coating. These coatings use binder systems which control polishing behaviour by different mechanisms. A broad range of binder technologies are found in this group and these have replaced TBT copolymer based paints which have been withdrawn from use. Binder systems range from those based on the dissolution of metal carboxylates and polymers relying on ion-exchange to polymers relying on hydrolysis to control the rate of polishing. Modification of the binder systems and pigment phases of products within this group can be used to tailor the products towards different end uses. The

Coating Type	Description, mode of action and properties
	requirements for protection of a fast moving and very active vessel can be very different from that of a slow moving less active one. Such modifications can also be used to tailor performance to accommodate the potential intensity of fouling. The different binder technologies can be used alone or in combination and result in products with varying levels of antifouling protection. Other binder components may also be added in order to modify the overall properties of the paint film. Typical dry-docking intervals for vessels coated with self polishing antifouling paints range from 24 to 60 months, however these systems may also be specified for lifetimes beyond this period.

Appendix 21. Published paper (CEPE Antifouling Working Group)

NOTE to the reader:

In the following CEPE methodology there are several issues that contradict with the requirements in the guidance document (e.g. number of trial panels, period of testing). The CEPE methodology can be used as long as the agreements of the guidance are respected.

TMI2013-PT21_efficacy_workshop-CEPE Efficacy Methodology for BPR - Revised 19 June 2012.doc

The European Council of producers and importers of paints, printing inks and artists' colours - CEPE
Guidance developed by the CEPE Antifouling Working Group

Efficacy evaluation of antifouling products

Conduct and reporting of static raft tests for antifouling efficacy

Specific scope

This document provides a baseline methodology for evaluating and reporting the efficacy of antifouling coatings. Efficacy is assessed by static raft testing relative to a negative control and, if used, a positive control coating. Efficacy may be indicative of, but has no direct one-to-one relationship with the actual performance of a product under real life conditions.

Document version

First approved in 2011-04.

Revised in 2012-06

1. Scope

Overview: The purpose of this document is to provide a methodology for determining efficacy of antifouling coatings by panel testing on static floating rafts. The document provides guidance on how to conduct, assess, record, and report results from efficacy evaluations.

Efficacy is evaluated relative to a suitable inert, negative control. A positive control of proven antifouling performance may also be included. This static exposure methodology for natural environments is not suitable for establishing absolute performance characteristics of antifouling coatings in service.

Objective: This methodology may be used by industry to obtain efficacy data during the development of new antifouling coatings. This methodology may also be used to provide national registration authorities with the information required to support the label claim of antifouling products. Efficacy is demonstrated when the extent of fouling is visibly less than on a blank panel.

The methodology is especially useful for:

- the persons responsible for writing the protocols for antifouling efficacy trials
- the persons responsible for conducting trials including the evaluation and recording of results
- the persons responsible for assembling and submitting dossiers for the registration of antifouling paints

- the national authorities which are responsible for the assessment of registration dossiers.

Reproducibility and accuracy: In static raft testing the fouling intensity will vary significantly between different geographical locations, between positions on the same rafts, and from season to season. More importantly, fouling will vary from one year to the next even for identical panels where exposure starts around the same date in different years. This variability in fouling intensity, and thus the test results, is due to weather conditions, availability of nutrients, and other uncontrollable factors that may affect the type and extent of fouling and its rate of settlement and growth. Therefore, the absolute amount of fouling present on the test coating and controls may not be reproducible at the same site from year to year.

Interpretation of results: The results obtained by this methodology demonstrate the ability of antifouling coatings to prevent settlement of fouling organisms under static conditions relative to a suitable negative control and, if used, a positive control tested simultaneously at the same site. An evaluation of the relative antifouling effect of an antifouling coating compared to the negative control and, if used, the positive control is used as a tool to indicate the potential of a tested coating to protect underwater structures. The results can be used to support appropriate label claims of the antifouling coating tested and to screen for new candidate products.

Efficacy testing on raft panels represents a worst case scenario compared to real life conditions. The main reason is that the exposure is static with limited opportunity for organisms to be removed by hydrodynamic forces. Ships' and boats' movement through water also aid the release of active ingredients from their antifouling. Furthermore, fouling intensity is generally recognised as being greater near the coast relative to the open seas.

2. Definitions

Antifouling coating: A material which, when applied as a surface coating, is used to control the settlement and/or growth of fouling organisms on submerged surfaces including ships, boats, aquaculture equipment, offshore oil installations, and other man made structures.

Negative control: An inert reference surface that does not control fouling (e.g. an anti-corrosive coating).

Positive control: A reference surface coated with an antifouling coating of appropriate efficacy relevant to the intended end use of the test coating.

Fouling season: The months of the year during which significant settlement and growth of fouling organisms typically occur on a negative control at the test site.

3. Apparatus

The following equipment will be required to undertake efficacy testing according to this methodology.

Panels: Panels are typically made of plastic (e.g. PVC), reinforced polyester, steel, aluminium, marine grade plywood, or other material suitable for extended immersion in natural waters. (Metal panels must be adequately protected with an anticorrosive paint system.)

Panels should be designed to allow them to be securely fixed to the test raft, for example via a suitable panel rack. Where the design requires fixing holes through panels, these holes should be drilled prior to the application of the coating to prevent damage.

The panels may be designed to allow one or more coatings and/or controls to be tested on each individual panel. The total immersed area of each coating or control should be no less than 100 cm^2.

Raft: A free floating platform which has been designed to allow test panels to be affixed and immersed at a constant depth in natural waters. The design of the raft should enable panels to be readily removed for inspection.

The minimum depth of water below the raft at low tide should generally be 2.5 m.

The floating raft should be of sufficiently rigid construction to withstand prolonged exposure to weather and wave action and prevent excessive flexing or movement of test panels. It should be designed to ensure the occupational safety of users.

The raft should be designed to ensure that all test coatings and controls of the same test series are exposed to similar levels of sunlight and water flow to minimise variation. To increase the testing capacity, panels may be affixed to the raft in rows at the same depth. Where relevant the spacing between parallel rows at the same depth should generally be at least 20 cm to allow sufficient water circulation and illumination.

Generally, the raft design should ensure that panels are fully and permanently immersed. Panels should normally be exposed vertically and at a fixed depth from 0-3 m below the water surface. The lower edge of the panel should always be at least 0.5 m above the sea bed.

The raft may also be designed to allow coatings that are intended for use in darker or lighter areas to be tested under relevant conditions where the coating receives less or more sunlight. In such cases panels may be mounted on the raft facing partly down or up. Shade may also be provided by covering parts of the raft.

4. Safety

This test methodology does not address possible safety, health and environmental concerns associated with its use. All operations should be performed in accordance with all relevant local and national regulations.

Personal protection: Antifouling coatings may contain hazardous materials that could cause skin and eye irritation on contact and adverse physiological effects if inhaled. Thus, application and drying should take place in a well ventilated area and appropriate personal protective equipment should be worn during application. Product safety data sheets should be consulted when available.

Environmental protection: Unused paint and other contaminated material as well as panels after exposure should be disposed of as hazardous waste.

5. Procedure

All controls and test antifouling coatings should be tested under equivalent conditions. The exposure (immersion) of controls and test antifouling should start simultaneously (around the same date) and the exposure should be at the same location at the same depth and orientation.

Panel preparation: The test coating and positive control should be applied to panels according to the manufacturer's guidelines to ensure adhesion during the period of the study. Appropriate drying and recoating intervals and temperature and ventilation requirements for application of the coatings should be followed.

An appropriate means of application should be used. Typical methods include spray, roller, brush, or specialised application equipment like a bar type applicator. Sufficient film thickness, taking the expected polishing and leaching rate characteristics of the product into account, should be applied to last for the planned duration of the test. Unless both sides of a panel are used as test substrates, the back of the panel may be

coated with an antifouling of proven efficacy to prevent fouling on the back. Edges may be painted with the coating under test or with a different coating of proven efficacy. All panels should be marked indelibly with a suitable reference code to aid identification.

Replicates: In cases where the purpose of the test is simply to demonstrate the efficacy of a test coating relative to a negative control, the use of single panels may provide data of sufficient quality. When replication is used, the number of replicates should be appropriate for the specific purpose of the test and should have the same orientation as the test panels and controls. Read-across to efficacy data from other test panels in a test series of similar formulations with the same content of active ingredients may also be used when justified and reasonable to support the results obtained for the test coating.

Exposure time: To verify efficacy, the minimum immersion time for testing is six months. In locations where the fouling season is shorter than six months this period may be reduced. The efficacy test should cover at least one continuous and complete fouling season where appropriate. Since raft panel exposure is static, fouling intensity is high, and the tests may be regarded as an accelerated test for products for vessels.

6. Evaluation

Frequency: Antifouling coatings under test and controls should be regularly inspected and evaluated for surface fouling, typically about every two months during the fouling season. Evaluations are not necessary during periods where there is minimal settlement and growth of fouling organisms (e.g. in cold and temperate regions where winter conditions do not support fouling settlement). Generally, the panels will be removed from the water for evaluation and, except at the end of the test period, returned to the water immediately after evaluation.

Rinsing: Optionally, panels may be rinsed gently with water from the site in order to reduce the influence of non-sessile organisms (that would be removed by low shear forces). Rinsing may also be carried out to remove possible sedimentary material (clay or silt). If utilised, rinsing must be performed on all panels equally and at each inspection. The method chosen, or if panels are not rinsed, must be specified in the final report.

Evaluation procedure: The type and severity of fouling that is present on the test coating and controls shall be assessed at each inspection. Evaluation may be made by visual assessment on site or any other appropriate method (e.g. image analysis). The three major types of fouling observed on the test coating or controls; Slime, algae, and animals, should be separately assessed since the same percentage of coverage may have very different economical penalties during actual in-service use (e.g. effect on the friction of a vessel through water). Also fouling organisms that are known not to attach on moving vessels, but may be frequent on static surfaces, should be assessed separately (e.g. amphipods).

Further classification of the fouling organisms present may, in addition to slime (biological film of microfouling including bacteria, diatoms, micro-algae, and extracellular biopolymers), generally be restricted to main categories such as green, red, and brown macro-algae, bryozoa, hydrozoa, barnacles, tube worms, ascidians, and mussels. A more detailed determination is generally not necessary since products shall prevent attachment of fouling irrespective of species (or other taxonomic ranking).

As the assessment is based on a visual inspection, it is advised that this is done by a trained operator. This will help to improve consistency and data quality.

Assessment for the severity of fouling for each type of organism should be semi-quantitative, for example using a scale from 0-4, where 0 indicates the absence, and 4 indicates complete coverage of the class of organism in question. Optionally an estimation of the percentage coverage can be used.

The assessment of the coverage of algae and other soft fouling (e.g. arborescent bryozoans, and hydroids), should be based on the area covered by the "hold fast" (the attached base of the organisms) and not by the area covered by the "fronds" (leaves of macro-algae) or offshoot colonies.

Overall fouling assessment: The individual assessments of the fouling coverage of each type of organism may be combined to provide an overall fouling assessment. To generate this, a weighting of the coverage of the different types of fouling may be applied to rate and characterise the severity of the fouling present.

When the coating under test is intended for use on ships, fouling never seen on active vessels (e.g. amphipods) may be disregarded during the weighting. Biofouling attached to other fouling organisms (secondary fouling) should also be excluded from the overall fouling assessment.

Only the fully immersed surface area (if parts of the panel are subject to splash only) should be included in the determination of the fouling rating. Fouling attached within 1 cm from all edges of the test panel and fouling around the cable ties/studs/etc. may be disregarded in cases where an edge effect is seen. (Fouling around edges is normally attributed to insufficient antifouling paint film thickness around sharp panel edges.)

Fouling caused by physical defects or damages in the substrate or accidental damages of the antifouling should be disregarded. Fouling on exposed anticorrosive paints or other substrates (except where these are used as negative controls) or on other antifouling paints that may be used to coat panel edges, should be excluded from the assessment.

Physical defects (detachment, blistering, cracking, etc.) attributed to the inherent properties of the antifouling paint itself should be recorded and reported.

Photos: Inspection reports should include panel photos from each inspection.

7. Reporting

The report should contain all relevant information obtained from the efficacy trial for a given product. This may include:

- The name of the reporting company (and client if the test is carried out on assignment)
- The geographical location of the test raft(s) (including longitude and latitude)
- The geography (e.g. open sea, bay, estuary, etc.), depth of water, and water exchange conditions (tide, currents) at the raft site
- Typical local conditions (e.g. water temperature, salinity, and pH at the raft site).
- Relevant information on the typical fouling community at the test site and seasonal influences where applicable.
- A discussion of any special conditions or variables that may have arisen particular to the specific test
- Orientation and exposure depth of test panels
- Dimensions and type (material) of test panels
- Identification of the tested product and control(s)
- Details on the panel preparation for the product under test and the control(s) (No. of coats, film thickness, application technique, etc.)
- Number of replicates if used
- Initial date of immersion and the cumulative exposure time (in months) for subsequent inspections
- Raw data from each individual assessment of a test panel

- The overall fouling assessment rating at each inspection during the exposure period
- Photos of test and control panels
- A systematic appraisal of the efficacy of the test product in relation to the negative control and, if used, any positive controls and the method by which that appraisal has been conducted
- A description of the reporting company's weighting system used to provide the overall fouling assessment rating
- A discussion on the validity and acceptability of the test result relative to the intended label claim for the product tested when commercialised [e.g. recommended use area (recreational yachts, ships' niche areas, ships' flat bottoms, ships' water line, etc.) protection time/dry-docking interval, fouling conditions in targeted markets, etc.].

An interpretation of the test data generated and a conclusion on the efficacy of the coating under test.

Appendix 22. Example of how an overall fouling assessment may be carried out for panel testing in marine waters

In order to assess panels out in the field, an effective and simple system is needed. Very detailed assessments of fouling coverage do not increase the quality of the test, as field conditions are highly variable and static raft tests can only provide an indication of products' real life performance.

Individual companies have different ways of assessing the coverage of the main categories of fouling into an overall description of the efficacy of test panels. However, the principles of the example should apply to most assessment systems. Transparency of how the overall assessment is carried out is important in order to evaluate an efficacy report.

The fouling coverage on raft panels will be assessed based on coverage intervals. Each interval will be recorded by a different 'rating'.

Table 60: Example of categorisation of fouling coverage into ratings from 0 to 4

Fouling Coverage (examples of company specific intervals for coverage of fouling)		**Rating**
Company 1	Company 2	
0-10%	0%	0
10-30%	>0-25%	1
30-50%	25-50%	2
50-80%	50-75%	3
80-100%	75-100%	4

As different fouling species can contribute to different impacts on a vessel (e.g. fuel consumption of a ship), the coverage ratings may be weighted in several ways to take this into account. The applicant may provide references to literature that provide more detail on the assessment and weighting factors[70].

Table 61: Example of weighting of ratings

Type of fouling	**Weighting (of ratings from 1-4)**			
	Trace (1)	**Slight (2)**	**Medium (3)**	**Heavy (4)**
Light slime	0	1	3	5
Dense slime	3	5	10	20
Macro-algae	5	10	30	50
Animals	5	10	30	50

A score may be calculated by adding up the weightings. In this example, that value is then subtracted from 100. Zero growth (apart from traces of light slime) gives the

[70] e.g. IMO MEPC/60/4/21, 2010 from IPPIC

fouling resistance rating 100 (100-0) and heavy fouling of both algae and animals gives the rating 0 [100-(50+50)]. The rating is then allocated to descriptions of the overall efficacy.

Table 62: Example of categorisation of overall efficacy

Fouling resistance rating	Efficacy
Company specific score intervals, each with a corresponding characterisation of the efficacy	Excellent
	Good
	Fair
	Poor

Description of types of fouling:

Slime: Bacteria, micro-algae, and protozoa.

Light slime is easily removed from the surface.

Dense slime is not easily removed from the surface.

Algae (weed): Green algae, red algae, and brown algae.

Animals: Barnacles, tubeworms, mussels, hydroids, and bryozoans.

RELATING COMPANY FOULING ASSSESSMENTS TO THE NORMS AND CRITERIA FOR PRODUCT AUTHORISATION.

When applying for authorisation of an antifouling product, the applicant should provide their overall fouling assessment of the product, together with the raw data and photographs/diagrams of the panel tests.

This guidance document only takes into account the percentage of macro-fouling on the raft panels as pass/fail criterion, not the classification in the applicant's assessment system.

As the percentage coverage per rating may differ between different company's assessment systems (see Table 60), some systems might not record 25 % coverage (the pass/fail criterion) in their rating system (e.g. in Table 60 Company 1 has a borderline at 30 % not at 25 %). Therefore, not only the ratings and end category of the product should be provided but also the raw data of the panel tests. The percentage coverage with macro-fouling per panel can then be identified from the raw data. This percentage is used to see if the product is sufficiently effective (i.e. <25 % macro-fouling).

Appendix 23. PT 22 active substances in the review programme

Table 63: PT 22 active substances in the review programme

Active Substance	RMS	CAS No
Formaldehyde	DE	50-00-0
Bronopol	ES	52-51-7
Iodine	SE	7553-56-2
Quaternary ammonium compounds, benzyl-C12-18-alkyldimethyl, chlorides	IT	68391-01-5
Quaternary ammonium compounds, benzyl-C 12- 16-alkyldimethyl, chlorides (ADBAC)	IT	68424-85-1
Quaternary ammonium compounds, benzyl-C12-14-alkyldimethyl, chlorides	IT	85409-22-9
Quaternary ammonium compounds, C12-14-alkyl[(ethylphenyl)methyl]dimethyl, chlorides	IT	85409-23-0
Polyvinylpyrrolidone iodine	SE	25655-41-8

Appendix 24. Assessment grid for tests on human bodies

This grid is for use in the assessment of the biocidal product itself, but not for assessing the overall embalming process with its hygiene and cosmetic aspects.

Number of the report:

Name and signature of the embalming professional:

Company:

Address of company:

1. General information

Date of the operation:

Place:

Type of place:

☐ Funeral parlour

☐ Morgue

☐ Establishment without a morgue (fewer than 200 deaths per year)

☐ Home or other (please specify):

Identification:

Gender: ☐ Male ☐ Female

Age:

Estimated weight (kg):

Estimated corpulence: ☐ cachectic ☐ thin ☐ medium ☐ stout

Adiposity: ☐ low ☐ medium ☐ high

Date of death (if known):

Date and time of treatment:

Body refrigerated: ☐ yes ☐ no. If "yes", for how long:
Temperature:

Causes of death (if known):

Therapeutic treatment (if known):

2. Preoperative examination of the body

Body intact: ☐ yes ☐ no, description:

Autopsy before treatment: ☐ yes ☐ no

Presence of external prostheses: ☐ yes ☐ no

Surgical intervention before death (if apparent or known): ☐ yes ☐ no

If yes, type of intervention:

Other visible anomaly(ies):

Decomposition: ☐ none ☐ commencing ☐ problematic

Rigidity: ☐ none ☐ minimal ☐ moderate ☐ problematic

Dehydration: ☐ none ☐ normal ☐ high

Lividity: ☐ none ☐ minimal ☐ moderate ☐ problematic, location:

Coloration of tissues (yellowing): ☐ no ☐ slight ☐ moderate ☐ intense, description:

Dermal lesions (sores, blisters, wounds, etc.): ☐ yes ☐ no, description:

Distension of the abdomen: ☐ no, ☐ slight ☐ moderate ☐ intense, ☐ liquid ☐ gas

Bruising: ☐ yes ☐ no, ☐ abdomen ☐ thorax ☐ leg, ☐ arm, ☐ face, specify degree and place:

Comments:

3. Techniques used for injection of the biocidal product

Time of start of treatment:

Time of end of treatment:

Site(s) of injection:

Carotid(s): ☐ right ☐ left.

Femoral(s): ☐ right ☐ left

Axillary(ies): ☐ right ☐ left
Other(s), description:

Ease of finding: ☐ easy ☐ normal ☐ deep

Condition: ☐ good ☐ atheromatous / ☐ hardened

Injection: ☐ manual ☐ by electric pump ☐ by gravity

Diffusion: ☐ good ☐ fair ☐ bad

Puncture before treatment: ☐ yes ☐ no. If "yes", type:
Biocidal product used:

Pre-injection: ☐ yes ☐ no

Injection:

Hypodermic: *product:*

Site:

Topical: *product:*

Site:

Name of the biocidal product:

Active substance(s):

Duration of efficacy claimed:

Number of litres:

Arterial fluid:

Name of fluid:

% of dilution:

Number of litres injected:

Start time for the injection:

End time for the injection:

Cavity treatment:

Name of fluid:

% of dilution:

Number of litres injected:

Start time for the injection:

End time for the injection:

Corrective injection:

☐ yes ☐ no

Drainage method:

☐ Cardiac, ☐ Venous

Vein(s) chosen: ☐ jugular, ☐ femoral, ☐ axillary

Volume drained by circulatory system (*litres*):

Total volume drained (*litres*):

Type of drainage: ☐ drain tube(s) ☐ forceps ☐ intermittent / ☐ continual

Quality of drainage: ☐ considerable clotting ☐ medium ☐ slight ☐ no clotting

General puncture:

Quantity*:*

4. Observations concerning the injection of the biocidal product

Observations during the treatment:

Odour: ☐ normal ☐ fair ☐ bad

Colouring: ☐ good ☐ fair ☐ bad

Suppleness of the skin: ☐ good ☐ fair ☐ bad

Observations following the treatment:

Odour: ☐ good ☐ fair ☐ bad

Colouring: ☐ good ☐ fair ☐ bad

Suppleness of the skin: ☐ good ☐ fair ☐ bad

Mandatory observation 48 hours after the treatment:

Odour: ☐ good ☐ fair ☐ bad

Colouring: ☐ good ☐ fair ☐ bad

Suppleness of the skin: ☐ good ☐ fair ☐ bad

Optional observation (at times relevant to the manufacturer's claims):

Time after treatment:

Odour: ☐ good ☐ fair ☐ bad

Colouring: ☐ good ☐ fair ☐ bad

Suppleness of the skin: ☐ good ☐ fair ☐ bad

Other products used during the preservation process:

Reasons for their use:

Description:

Products for cosmetic purposes:

☐ yes, ☐ no. If yes: ☐ normal, ☐ make-up, ☐ significant, ☐ restorative

Other restoration: _____

Moisteners and other products used (cauterising agents, disinfectants, skin tone correctors, etc.):

Name of the fluid: _____, % dilution: _____, litres injected: ___

Name of the fluid: _____, % dilution: _____, litres injected: ___

Explanations:

5. Comments

Appendix 25. References to Chapters PT 11 and PT 12

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Appendix 26. Definitions for PT 11 and PT 12

Term	Explanation
Blowdown water	Blowdown water corresponds to the fraction of water that is drained from cooling equipment while simultaneously replacing it with make-up water. This process dilutes the mineral concentration of the system water that steadily increases due to water evaporation.
Clear water or filtrate, superclear filtrate	The filtration process of white water in papermaking process generates different quality of filtrates. Further steps of filtration provides good and high quality filtrates; they contain much less suspended solids. Clear/superclear filtrates may be used in spray application such as shower water (filter cake removal) or discharged.
Cloudy water or filtrate	The filtration process of white water in papermaking process generates different quality of filtrates. Initial step of filtration providing "low" quality filtrate: it still contains significant amount of suspended solids. It is used for the stock preparation.
Drill cuttings	Pieces of rock cut or crushed by the drill that come out of the well (a deep hole made in the ground, to locate oil) whilst a well is being drilled to an oil or gas reservoir. The cuttings will be mixed with drilling fluid which is used (among other things) to lubricate the drill.
Drilling fluids	A number of liquid and gaseous fluids and mixtures of fluids and solids are used in drilling operations often termed "drilling mud". They are used to provide hydrostatic pressure in the well during drilling, keep the drill bit cool and clean, and carry drill cuttings out of the well suspended in the fluids. Under OSPAR drilling fluids are classified as either water-based muds (WBMs), synthetic fluids, organic-phase drilling fluid (OPF) or oil-based fluids (OBF).
Drilling muds	See drilling fluids.
Dry broke	Paper left over from for example converting, inferior quality paper produced by the paper machine which needs to be reworked; the dry broke is repulped in a broke pulper and goes to the mixing chest in the papermaking process.
Export fluids	Oil or natural gas liquids that are exported from the platform to the refinery.
Holding time index	The "Holding Time Index" (HTI) is the half-life of a substance in a system. This parameter indicates the time required for a given chemical dissolved in the system water to reach 50% of its initial concentration. The HTI is calculated as HTI=0.693 × V/BD, (V= system volume, BD= Blowdown rate).
Hydraulic cycle time	Hydraulic cycle time corresponds to the duration needed for the cooling fluid to do one cycle in the system. The time of cycle is calculated as volume of recirculated water /recirculation flowrate. Note: in case of once-through cooling systems, the hydraulic cycle time corresponds to the duration between the entrance and the exit of the fluid from the system.

Term	Explanation
Inter-platform pipelines	A tube or system of tubes used for transporting crude oil and natural gas from one platform to another prior to export to a refinery. This may be done to consolidate the streams prior to export.
Make up water	To compensate for the water losses in the circuit (e.g. by evaporation loss, drift loss, by blowdown and due to leakage loss), makeup water is added to the sump.
Off-line system	In the context of cooling system, off-line system means that the system is not operated due to implementation of a cleaning and/or disinfection event. As an example, stopping of the heat source or cooling fans in cooling systems in order to clean and/or disinfect the system would be considered as an off-line operation.
Produced water	Water that comes out of the well with the crude oil or natural gas during production. Produced water contains soluble and non-soluble oil/organics, suspended solids, dissolved solids (see TDS above), and various chemicals used in the production process.
Raw water	Source of water such as a river or a bore hole with or without a filtration treatment or biocide treatment.
Recovered fibre	Fibres/pulp recovered from a disc filter or from a DAF/Krofta (device where fibres are made to float for separation from the clarified water).
Shower water	Clear or super clear filtrate or a mixture of clear or super clear filtrate with fresh water or just fresh water. Used for spray applications.
Slime	A slippery deposit found in papermaking process defined as slime. This deposit or biofilm consisting of an accumulation of extra cellular polymeric substances (EPS), bacteria, mould and/or yeast and may contain pulp fibre, alumina, filler, carbonate, TiO_2, ferrous particles, clay etc.
Thick stock	A suspension of paper pulp and other material from pulper to machine chest, the mass fraction of solids is 2 to 5%
Thin stock	Dilution of thick stock made with white waters, the mass fraction of solids is from 0,1 to 1,5%. The thin stock is sent to the headbox to form the paper.
Total Dissolved Solids (TDS)	In formation water analysis, TDS is the soluble components in a sample, or the residue left after evaporation of a sample, reported as ppm or mg/L. These are dissolved salts from the reservoir.
Total fluids	Oil and gas reservoirs contain a mixture of oil, gas (natural gas condensate), and water, and when these are pumped or "produced" from the reservoir the mixture is termed the "total fluids". Which will then go through various separation processes.
Wet broke	Remaining pulp from the papermaking process after the formation of the sheet, for example in case of the pressed paper sheet does not get onto the drying section.

Term	Explanation
White water	Water that is drained from paper as the sheet is being formed. It contains pulp fibres and fillers, such as clay or calcium carbonate. Recycling of white waters is achieved by direct recirculation (thick stock dilution) and after filtration in order to be properly reused in the process. The filtration process generates different quality of filtrates: Cloudy water or filtrate, clear water and shower water.

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